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DEPARTMENT OF PHYSIOLOGY OF COLUMBIA UNIVERSITY

1922-1926

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L'ACTION DE L'ALCOOL ISOPROPYLIQUE SUR LA FORCE FONCTIONNELLE DU COEUR.

PAR

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Permettez-moi d'abord de vous remercier de l'honneur que vous avez bien voulu me faire en m'invitant à contribuer au „Livre jubilaire" de Monsieur le Professeur ZWAARDEMAKER. Ce m'est un grand privilège d'ajouter à ce volume le résultat de mes humbles connaissances scientifiques, en témoignage d'estime à notre éminent collègue, le Professeur de Physiologie de l'Université d'Utrecht.

J'ai fait récemment une série d'expériences sur l'action de l'alcool isopropylique comparée a celle de l'alcool éthylique. Cette étude peut bien être choisie pour l'occasion, parce que son étendue se conforme au nombre de pages assigné aux différents collaborateurs de ce volume. L'alcool isopropylique, ou alc. propylique secondaire, a été employé récemment comme remplaçant pour l'alcool éthylique dans certaines préparations cosmétiques. Il faut se rappeler que c'est un homologue de l'alcool éthylique et qu'il a la formule empirique $C_3 H_7 OH$. Il est isomérique avec l'alcool propylique normal, mais il en diffère en ce que son groupement hydroxyle est combiné avec l'atome central des trois atomes de carbone et non pas avec l'un des extrémités.

Quoique j'aie fait plusieurs expériences comparatives au sujet de l'action de l'alcool isopropylique et de l'alcool éthylique injectés dans le courant circulatoire veineux chez des chiens, j'ai cru prudent de répéter ces expériences selon une méthode qui nous permet de constater les changements circulatoires d'une manière plus plastique que selon la première. Les chats employés pour cette étude ont été maintenus dans un état de narcose légère sous éther pendant toute la durée de ces expériences. Après que le côté droit de la poitrine eut été ouvert, on a inséré

un „Stromuhr" ou mesureur de courant dans la veine cave inférieure tout près de l'oreillette droite. [1]) On a enregistré la pression artérielle générale avec un manomètre à mercure, tandis que la pression veineuse était enregistrée par un manomètre à membrane, qui communiquait avec le stromuhr par une voie latérale de la canule centrale. Cette voie latérale était ajustée de manière qu'une aiguille hypodermique pouvait y être insérée à tout moment pour injecter l'alcool. L'indication du temps par un chronographe Jacquet a servi d'abscisse pour toutes ces mesures.

En résumé, ces expériences avaient pour but d'enregistrer le courant circulatoire normal dans la veine cave en rapport avec les pressions artérielle et veineuse, avant et après qu'une quantité définie d'alcool isopropylique ou d'alcool éthylique eut été injectée par la canule latérale. Les injections ont été faites lentement et furent répétées plusieurs fois pendant le cours de chaque expérience. On a employé principalement le même type d'alcool pour les épreuves successives, quoiqu'on ait employé aussi ces alcools l'un après l'autre.

Le fait d'administrer 3 cm^3. d'alcool isopropylique en dilutions de 10, 25 et 50 pour cent n'a pas altéré considérablement la force fonctionnelle du coeur. S'il y avait quelque diminution, elle était légère et de nature temporaire. On a obtenu à peu près les mêmes résultats avec de l'alcool éthylique.

L'alcool isopropylique en dilution de 75 pour cent aussi bien que non dilué à causé des dépressions vasculaires bien définies, mais dont la restauration a été rapide. L'alcool éthylique, quand on l'a administré en quantité et force égales, a produit une dépression pareille, quoique plus faible. Malgré tout, ces résultats ne justifient pas la conclusion que l'alcool éthylique soit décidément moins toxique que l'alcool isopropylique. Macht[2]) donne comme dose mortelle d'alcool éthylique 5 cm^3. par kilogramme de poids du corps et 2.5 cm^3. par kilogramme comme dose d'alcool isopropylique. Ces résultats, que l'on base sur les effets d'injections intraveineuses, indiqueraient que l'alcool isopropylique est deux fois plus toxique que l'alcool éthylique. La dose mortelle minimum pour les chats, administrée par la bouche, est fixée à 6 cm^3. par kilogramme de poids du corps. [3]) Chez les lapins, la dose léthale minimum

[1]) On a employé le Stromuhr décrit par moi dans *Pflüger's Archiv*, 1908, CXXI, 150.

[2]) *Jour. Pharm. and Exp. Therap.*, 1920, XVI, 1.

[3]) *Arch. intern. de Pharmacol. et Thér.*, 1921, 285.

de 1.5 mm. Hg. Trois secondes après le commencement de l'injection, la pression artérielle a diminué jusqu'à ce qu'elle atteignit la valeur de 74.2 mm. Hg., 15 secondes plus tard environ. La pression veineuse est montée légèrement pendant la période toute entière. En coincidant avec la descente de la pression artérielle, l'écoulement veineux dans le cœur a diminué de 1.23 cm^3. par seconde, ce qui indique une réduction certaine de la force fonctionnelle du cœur. On peut confirmer l'hypothèse que cet effet peut être localisé dans l'organe, par la brièveté de la période qui s'est écoulée entre le commencement de l'injection et l'accès des phénomènes qu'on vient de décrire. On peut la confirmer encore par la nature des changements dans la pression du sang. On vient de dire que la pression veineuse monte tandis que la pression artérielle descend, ce qui montre une certaine impuissance de la part du cœur d'émettre des quantités normales de sang. L'existence de la stagnation est suggérée d'ailleurs par une réduction bien décidée de la fréquence de cet organe et par une force particulière de ses pulsations individuelles. Ainsi, on constate un taux normal de 174 par minute contre un taux de 115 par minute après l'injection.

Ces dépressions vasculaires ont disparu bientôt après l'injection et des conditions dynamiques normales ont été rétablies en une demi-minute après l'administration de l'un ou l'autre alcool. Ces injections ont pu être répétées plusieurs fois sans résultat fatal, pourvu que les intervalles entre les administrations successives ne fussent pas abrégés outre mesure. Dans beaucoup de cas, les animaux qui avaient reçu plusieurs doses d'alcool éthylique ou isopropylique, présentaient une résistance décidée, comme l'a montré le fait que la respiration artificielle a pu, dans ces cas, être arrêtée pendant longtemps, sans que le cœur montrât aucune tendance diastolique dangereuse.

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THE CONCENTRATION OF SUGAR IN THE BLOOD OF THE RABBIT DURING INANITION AND AFTER THE INGESTION OF GLUCOSE

ERNEST L. SCOTT and THOMAS H. FORD

From the Department of Physiology, Columbia University, New York

Received for publication November 23, 1922

The dog has become, perhaps, the standard laboratory animal for the study of the sugar in the blood, and it may be justifiably so. Before generalizations are made on the basis of such studies it is, however, necessary to remember that the dog is naturally a strict carnivore—in the wild state even an obligate carnivore—and that its mechanism for handling the carbohydrates may be quite different from that of the omnivores and herbivores which ingest much larger quantities of this type of food.

While the rabbit is the most available herbivore which is in common use in our laboratories, it has been rather generally discarded for this type of work because of the feeling that it is very susceptible to emotional hyperglycemia and glycosuria (1), (2). On the other hand we have the statement of Jones (3) that successive determinations of blood sugar upon individual rabbits were constant within the experimental error of the method used for analysis. This statement encouraged us to use the rabbit in the present research. While our results would not warrant us in making the above statement, we believe that they are sufficiently uniform to justify us in drawing certain conclusions which may be of use in the study of carbohydrate metabolism.

Methods: *a. Chemical.* The method proposed by MacLean (4) for the determination of sugar in blood, modified as noted below, was used throughout the work. The modification consisted in the substitution of phosphotungstic acid as the protein precipitant in place of the one originally described. This necessitated the preparation of a new conversion table, but as no particular difficulties were encountered, and as the reasons for the modification will be discussed elsewhere, the method will not be further described at the present time. The modified method was checked in the usual ways and was found to recover glucose satisfactorily from a solution in water, from blood which

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had been continued at 37°C. until it had lost all reducing power for the copper solution and to which glucose in known amounts had then been added, and from fresh blood (to which varying known amounts of glucose had been added).

A few determinations of the glucose in human blood were made in parallel by the modified Maclean method and by the method of Folin and Wu. These determinations gave identical results within the limits of error of the methods.

Duplicates run by the modified method seldom differed by more than 5 mgm. per 100 cc. of blood. With the exception of some half dozen determinations where it was not possible to obtain sufficient blood, or in which one of the duplicates was lost during the analysis all determinations were run in duplicate and the results reported are the means of the duplicates.

A. Fasting.—All of the rabbits included in the report were in a healthy condition and usually gained in weight while they were under observation. In none of the cases reported was there a significant permanent loss in weight. They were fed once daily on a mixture of grain and green vegetables, usually oats and cabbage. The feeding time was about 5 p.m., except on experimental days when it was postponed for the rabbits serving as subjects for an experiment until after the last sample was drawn. As the experiments were begun between 10 and 12 in the morning, it will be seen that the rabbits were subjected to an absolute period of at least 24 hours before drawing the initial sample of blood.

When glucose was fed experimentally, it was given in solution by mouth from a metal-tipped pipette. Though it was necessary to discard an occasional animal because it would not take the sugar in this way, it was found that as many rabbits could be administered without appreciable loss, and we felt that it was much less disturbing to the animal than the use of a stomach tube.

The blood was drawn from the heart and was arterial except in the cases noted in the tables. Occasionally hemorrhage into the pericardium would occur, but we have taken care that no case reported is included in the present paper.

In studying the alimentary glycosuria five samples were obtained, as a rule in almost quantitative agreement. The first just before administering the glucose, then at the half hour, hour, and at 2 and 3 hours after feeding. The results of our work would not indicate that any change in these times is desirable, though it might be instructive when it is possible to increase

the number of samples, to extend the time somewhat, especially when large amounts of glucose are fed.

c. Statistical. Because of the considerable variation in the concentration of sugar which we found in fasting normal animals, we deemed it desirable to introduce some of the simpler statistical conceptions into our treatment of the results. If the reader is not familiar with the significance of the terms which we have used, he is referred to King (6) or to Yule (7).

Experimental: *a. The sugar present in the blood of the rabbit during inanition.* Because it is so generally believed that the rabbit is not fitted for work of this kind, it was necessary for us to justify its use experimentally before attempting the feeding experiments. This we endeavored to do by determining the sugar in the blood of the rabbits which had been prepared as for the first sample of an experimental series. That is, they were first kept quietly in the laboratory for a few days upon the standard diet. Then after an inanition period of about 24 hours, a sample of blood was obtained and the sugar determined. Only the first sample obtained from a given rabbit in any one day was included in this series. It was, however, thought legitimate to extend the series by adding to it the initial samples obtained in the feeding experiments. These latter constitute about 40 per cent of the entire series.

Though our data bearing upon emotional glycemia in the rabbit are not included in the present paper, it may not be out of place to state that our experience has been that fright is usually, though not always, accompanied by hyperglycemia, and that the degree of hyperglycemia does not necessarily follow the *apparent* emotional disturbance. So far as it is possible to judge, the rabbit does not show as great an increase in glycemia for a given degree of excitement as does the cat. In this connection it should be remarked that the most common emotion in the cat is anger, rather than fright as in the rabbit.

No observations in which the animals did not conform strictly to the standard conditions as outlined above, as well as none in which the rabbit was recorded as being frightened or excited are included in this group. When the work is limited in this way, we have a series of 85 observations upon 27 animals which may be taken as being representative of what may be expected in normal unfrightened rabbits that have been subjected to an inanition period of about 24 hours.

The arithmetical mean for the series is 118 mgm. per 100 cc. of blood. The median is almost the same, 116 mgm. per 100 cc. As a means

of measuring the dispersion we have adopted the standard deviation as being the most satisfactory. This is represented by the following formula: $\sigma = \sqrt{\frac{\Sigma(d^2)}{n}}$ where σ = the standard deviation, d = the arithmetical difference between the individual observations and the mean for the series and n = the number of observations.

The calculated deviation for this series is 21.5 mgm. This gives a range of deviation from 64 to 150 mgm., within which range one would expect the major portion of the observations in any such series to fall. Also, and following from this, one would not safely state that a change in the glycemia observed during the course of an experiment, was due to the imposed experimental conditions unless it differed from the mean of the control by more than twice the calculated deviation, or better, by the sum of the standard deviations of the control series and of a series of animals subjected to conditions similar to those of the experiment. That is, should the range of standard deviations of an experimental series overlap that of the control series at all, many overlap it to any considerable extent, general conclusions could not be drawn. Enough modifications in a certain direction may result in a certain proportion of the cases examined.

The results have been plotted in the form of a frequency curve which is shown in figure 1. In the same figure are shown the position of the mean, the median and the range of the standard deviation.

In figure 2 are shown chronologically the concentration of sugar in the blood of a few individuals which were under observation for a considerable time. The figure makes it clear that it is not safe to depend upon experiments as yielding data when the controls are made upon the same animal, unless there has been a considerable number of controls as well as of experiments, and for purposes of generalization these would be preferable if made on a number of individuals.

Since rabbits with identical larvæ in the vagus centers of initial energies of tissue when the animals have been standardized as carefully as is practicable, we should expect an even greater divergence in their individual responses to experimental conditions which have been imposed upon them. That this is the case is evident when the ranges of standard deviation for the various series shown in figure 2 are compared. From this figure it will also be seen that in a general way the degree of dispersion increases with the degree of modification from the controls. Thus in series 2, 3 and 4 the greatest standard deviation occurs at the time of highest glycemia.

b. Alimentary glycemia in rabbits. Altogether four series were run. Series 1 served as a control and was in every way similar to the others except that no sugar was administered. To each rabbit of series 2 was

Fig. 1. Frequency curve showing the distribution of different concentrations of glucose in the blood of rabbits over the range of occurrence.

given 1 gram of glucose per kilogram of body weight, with a variation in dosage of ± 0.2 gram to each of series 3, 2 grams ± 0.3, and to

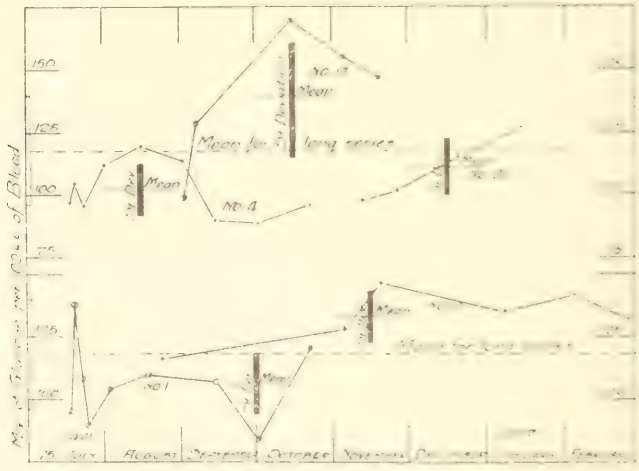

Fig. 2. Showing chronological variations in the concentration of sugar in the blood of individual rabbits. The standard deviation and mean for the long series are included for comparison.

each of series 4, 4 grams with a variation of ± 0.8 gram. The means for each period were determined separately for each of the different series, and the standard deviation about each mean calculated. These

TABLE 1

Circulation Data of Journals in Local Hospitals Not Found in Union Lists

TABLE 2

The control series. No sugar was fed

EXPERIMENT NUMBER	DATE	RABBIT NUMBER	BODY WEIGHT	TOTAL GLUCOSE FED	GLUCOSE FED PER KILOGRAM OF BODY WEIGHT	SUGAR PER 100 CC. OF BLOOD					REMARKS
						Initial	Hours after initial				
							½	1	2	3	
	1921		*kgm.*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
47	9–14	1	2.00	—	—	107	105	114	120	120	
48	9–14	4	1.80	—	—	90	94	94	102	107	
67	11–26	18	2.05	—	—	118	114	111	98	98	
68	11–26	20	2.50	—	—	102	105	102	110	110	
	1922										
71	1–7	21	2.66	—	—	123	152	140	171	150	
83	6–3	26	1.65	—	—	100	114	115	116	116	
84	6–3	27	1.55	—	—	114	127	114	89	125	
Mean						108	116	113	115	118	
Standard deviation						±10.6	±17.6	±13.2	±24.8	±15.4	
Adjusted mean; correction +10						118	126	123	125	128	

TABLE 3

Sugar in the blood of rabbits after feeding 1 gm. (±0.2) of glucose per kilo of body weight

EXPERIMENT NUMBER	DATE	RABBIT NUMBER	BODY WEIGHT	TOTAL GLUCOSE FED	GLUCOSE FED PER KILOGRAM OF BODY WEIGHT	SUGAR PER 100 CC. OF BLOOD					REMARKS
						Initial	Hours after feeding				
							½	1	2	3	
	1921		*kgm.*	*gms.*	*gms.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
38A	8–29	1	2.05	2.0	1.0	130	234	159	125[1]	114	[1] Venous blood
58	11–5	12	1.43	1.3	0.9	128	191	152	127	140	
59	11–5	13	2.22	2.0	0.9	155	250	198	177	128	
60	11–12	18	2.20	2.0	0.9	105	125	125	100	96	
61	11–12	19	1.96	2.0	1.0	119	164	127	98	90	
62	11–12	20	2.40	2.4	1.0	98	145	141	100	110	
70	12–20	19	1.70	2.0	1.2	140	173	195	158	116	
Mean						125	183	157	126	113	
Standard deviation						±18.2	±42.1	±27.6	±28.7	±16.0	
Adjusted mean; correction −7						118	176	150	119	106	

TABLE [illegible]

Sugar in the blood of rabbits after feeding 8 grams [illegible] of glucose per kilo of body weight

Rabbit number	Date	[illegible]	Body weight	[illegible]	[illegible]	Fasting	[illegible]	[illegible]	[illegible]	[illegible]	Remarks
23	9-1	4	1.97	8.0	7.4	112	207	166	166	141	
24	8-5	1	2.00	8.0	5.1	104	230	203	162	152	
28A	9-10	2	1.98	8.0	3.4	122	274	193	195	140	Venous blood
30	8-16	3	1.77	7.5	9.0	117	235	153	204	110	
31	8-16	4	1.72	8.0	3.5	136	220	202	154	129	Venous blood
32	8-18	4	1.77	8.3	3.0	110	199	189	172	130	Venous blood
39	9-1	4	1.73	7.0	3.3	114	200	219	149	99	Venous blood
44	9-7	15	1.80	8.0	2.2	129	167	167	140	109	Venous blood
45	9-8	17	2.00	8.0	4.8	105	142	182	126	170	[illegible]
50	10-1	1	1.90	8.5	2.1	89	140	209	69	128	Rabbit became very much excited
51	10-7	17	2.45	8.0	2.1	77	170	164	154	109	
54	10-15	13	2.10	9.40	2.4	170	312	295	182	110	
78	2-26	12	1.84	1.0	2.2	102	206	174	140	125	
Mean						115	198	187	150	131	
Standard deviation						±21.5	±40.8	±31.2	±29.4	±21.7	
Adjusted mean; correction +3						118	201	190	153	134	

TABLE 5

Sugar in the blood of rabbits after feeding 4 grams (±0.8) of glucose per kilo of body weight

EXPERIMENT NUMBER	DATE	RABBIT NUMBER	BODY WEIGHT	TOTAL GLUCOSE FED	GLUCOSE FED PER KILOGRAM OF BODY WEIGHT	SUGAR PER 100 CC. OF BLOOD					REMARKS
						Initial	Hours after feeding				
							½	1	2	3	
	1921		*kgm.*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
53	10–15	14	1.37	5.0	3.6	137	187	293	173	180	
63	11–19	12	1.46	5.0	3.4	145	220	218	185[1]	152	[1] Some difficulty in obtaining the blood; this sample not obtained; value given mean of 1st and 3rd hour
65	11–19	13	2.44	9.0	3.7	147	253	267	207[1]	147	[1] Rabbit became excited; 2nd hour sample not taken; value given is the mean of 1st and 3rd hour samples
66	11–26	19	2.00	8.0	4.0	118	222	189	156	117	
69	12–20	20	2.33	7.5	3.2	115	164	192	150	134	
	1922										
72	1–7	12	1.53	5.0	3.3	135	294	282	258	152	
76	2–4	21	2.98	10.5	3.5	130	263	254	167	170[1]	[1] Some difficulty in obtaining this sample; rabbit apparently not excited, but several punctures necessary
80A	3–18	24	2.90	10.0	3.5	147	204	238	184	197	
81	3–18	25	1.40	6.0	4.2	140	200	204	150	120	
82	3–27	26	1.66	6.5	3.9	143	204	211	204	152	
Mean						136	221	235	183	152	
Standard deviation						±10.9	±36.8	±35.7	±31.5	±23.9	
Adjusted mean; correction −18						118	203	217	165	134	

values are shown in tables 2 to 5. The adjusted means shown in the last line of each of the tables were obtained by adding algebraically to each time mean of the series an amount which would make the mean for the initial period equal to that of the control series shown in table 1, i.e., 118 mgm. per 100 cc. The values so obtained were plotted and the curves resulting are shown in figure 3. In this way the curves all originate in the same point, and the changes due to the feeding of the sugar are not confused by slight variations in the initial samples.

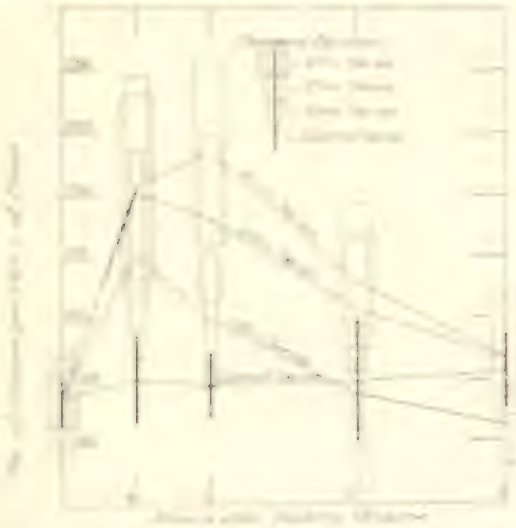

Fig. 3. Showing the effect of feeding various amounts of glucose upon the concentration of this sugar in the blood of rabbits.

A study of figure 3 reveals that: *a*. The range of standard deviations for the initial determinations of the different series is in no case in excess of that for the long series. The initial condition of the rabbits may then be presumed to be at least as uniform in each of the experimental series as it was in the longer series, and so the different series may be considered as being fairly comparable among themselves.

b. The control curve lies reasonably near the horizontal, and none of its ranges of standard deviation is excessive, nor do any of them exceed the ranges for the other series at the respective intervals, so that it may be presumed that the operation of drawing the blood is not a factor in the modifications which occur in the experimental series, and that it is legitimate to take this series as a basis for comparison.

2. At the end of the first half hour: *a.* The position of the mean for the 1-gram series is well above that of the controls, and while the range of standard deviation overlaps that of the control series to some extent, it lies wholly above the mean for that series and extends far above its upper limit of deviation. There would seem then to be little question that the administration of this amount of glucose will in most cases cause a considerable rise in the concentration of sugar in the blood at the end of half an hour; though there will be a few cases, say one in five, which would give values of the same order of magnitude as would be expected in fasting individuals.

b. The position of the means for the 2- and the 4-gram series indicates a still greater rise, and here even the lower limits of the ranges of standard deviation are well above the upper limit of that of the control, i.e., there is no overlapping. From this we would expect to find very few animals in either series giving values which could be confused with those given by control animals. On the other hand, one could not so surely distinguish between these two series and the one gram series, as here there is considerable overlapping.

c. The concentration of sugar in the blood drawn at this time would give no information as to whether 2 or 4 grams of glucose were fed, as in these cases the overlapping of the ranges of deviation is complete and the means are practically identical.

3. At the end of an hour the mean for the 1-gram series has fallen markedly, and its range of standard deviation now extends below that for the control series. We would now hardly expect more than two observations in three to be distinguishably higher than the controls. The means for the 2- and the 4-gram series have separated somewhat, the former having fallen slightly while the latter continued to rise. Their ranges of standard deviation are even farther above that of the control series than they were at the end of the half hour. There should, consequently, be little confusion between animals to which these amounts of sugar had been fed and fasting animals. In the same way there should be little confusion between the 1- and the 4-gram series. On the other hand it would not be possible to certainly distinguish individuals of the 2-gram series from either of the others to which sugar had been fed.

4. At the end of 2 hours the mean for each of the three experimental series has fallen markedly; that for the 1-gram series being even below that for the controls. This taken together with the position of the range indicates that while under these conditions there may be a

slight hypoglycemia (it is to be noted that it would not be revealed in isolated observations and the series may be considered as having returned to normal. The means for the other two series have fallen even more rapidly than that for the 1-gram series and of the two that for the 4-gram series has fallen the more rapidly. Overlapping has increased among all of the series and consequently the possibility of distinguishing between the series in isolated observations is becoming very small.

5. After 3 hours the mean for the 1-gram series has fallen far below that of the controls, and one which of the range of standard deviation may lie below that of the controls that one might hope to distinguish about 80 per cent of such animals from the controls at this time by a *hypoglycemia*. The other two series are apparently approaching a similar condition.

DISCUSSION AND INTERPRETATION. Currently in alimentary glycemia two factors are involved. These are: 1. the absorption of glucose from the intestine and 2. its elimination from the blood stream. It is also evident that the condition of glycemia at any given moment depends upon the relative rates at which these two processes are taking place. If the absorption is the more rapid, there will be an increasing glycemia while the reverse will be true if elimination is the more rapid, and if the two are going on at equal rates the glycemia will be maintained at a constant level.

From the general laws of physical chemistry it is probable, other things being equal, that the rate of absorption of a given substance will increase as its concentration in the intestine rises above that of the blood. From this, a rapid initial rate of absorption would drive up the concentration in the blood and so the rate of elimination. There probably would come a time, however, when the rates of the two processes were equal and the glycemia would be maintained for a longer or shorter time at a relatively high and constant level. Matters do not seem to be quite so simple as this, however, for while, as would be expected from the above, the level after half an hour is higher when 2 grams have been fed than it is after 1 gram, there is no difference in the level assumed at this time by the 2- and the 4-gram series. It is hard to see why the rates of removal from the blood should not be the same in these two cases, for the degree of glycemia was found to be the same and, from the plan of the experiment, the general body conditions of the animals must be assumed to be the same. On this basis there could have been no difference in the rates of absorption, and so one is driven to the conclusion that there is a definite upper limit to

the rate at which glucose can be absorbed from the intestine, regardless of the amount ingested. The mechanism of this is probably bound up with the facts revealed in Heidenhain's classical experiment, but we will not enter further into this now as it is the fact rather than the mechanism in which we are interested. Judged from the present experiments this limit, for the rabbits studied, was reached when somewhat less than 2 grams of glucose per kilogram of body weight were fed.

If the rate of absorption is no more rapid after feeding 4 grams than it is after 2 grams are fed, we should expect the maximum rate of absorption to extend over a longer period for the former than for the latter case, as there would be a larger supply in the intestine and so the intestinal concentration would be maintained above the minimum required for the maximum rate of absorption for a longer period. If, now, the rates of absorption and elimination from the blood have not come into equilibrium, after half an hour, sugar would continue to accumulate in the blood until such a time as equilibrium did occur. This is illustrated in the 4-gram series and by the "delayed maximum" found in normal men when large doses of glucose are given, and which is said by some to resemble the diabetic curve.

According to the proposed interpretation, the rate of absorption will fall, and at an increasing rate, as the intestinal concentration is reduced below the minimal amount required to maintain the maximum rate of absorption. Removal of sugar from the blood will, for a time, take place somewhat more rapidly than absorption and the glycemia will be reduced, provided the store houses have not become overloaded. In the case of diabetics, the store houses are to a greater or less degree eliminated functionally, so that elimination from the blood must take place largely or wholly through the kidneys, and so the process is delayed. This in turn delays the absorption and results in the long-maintained high level shown in the typical diabetic curve.

From the above it follows that the higher the amount of sugar fed and the consequent higher glycemia, the more rapid will be the fall after it once begins. This is illustrated by the curves shown in figure 3.

At first thought it might appear that this is not in accordance with what Strouse (8) implies when he likens the curves obtained from normal men who have received heavy doses of glucose to the diabetic curve and says that the difference between normal and diabetic men is only quantitative and not qualitative. However, it is quite possible that, had the curve for the men been studied after it had begun to fall, the rapid decline would have been as evident as it is in the case of the rabbits.

The most striking feature of the second- and third-hour periods is the very considerable hyperglycemia which is developed in the fasting rabbit during this time. While this is not a new observation (9, 10, 11), it is more strikingly shown in the series than is usually the case. We offer the following as a possible explanation of this phenomenon. During the period of hyperglycemia due to rapid absorption of glucose, the liver tissue may be considered as having become surcharged with glycogen and glucose. This would include the muscles and other tissues of active tissues of energy as well as the liver. Now it is conceivable, the degree of glycemia is ordinarily determined by the requirements for glucose of the tissues in which oxidation is taking place, a low glycemia would be expected in this case because of the plentiful store already present in these tissues. Though this explanation differs considerably from the one proposed by Macleod and deWesselow it is in essential agreement with the one proposed by Lüdin and Berglund.

It is the usual clinical practice in alimentary glycemia tests to administer 100 grams of glucose regardless of the weight of the subject. Since adult individuals in a normal condition of flesh vary from less than 50 to nearly 100 kilograms it results that in following this practice anything from 1 to 2 grams per kilogram of body weight may be fed. From figure 3 it will be seen that, at least for rabbits and on the basis of the above interpretations of the results, this covers a range of great variation in the rate of absorption and consequently a considerable variation in the form of the curve in individuals of different weights due to this factor alone. This variation in the curves would be confused with variations due to functional disturbances and should be eliminated if possible. This it seems to us could be done if a standard dosage per kilogram of body weight were substituted for the usual standard dose for an individual regardless of weight. It would further appear that the ideal for such a dose would be slightly more than enough to give the maximum rate of absorption. For the rabbit, 2 grams per kilogram would seem to be a good index for safety.

SUMMARY

1. It is desirable to use herbivorous as well as carnivorous animals in the study of carbohydrate metabolism. And though rabbits may not be as consistent as dogs, and perhaps more in their blood sugar content, nevertheless much may be learned from them if their variations are properly taken into account.

2. The concentration of sugar in the arterial blood of normal fasting

rabbits, when determined by MacLean's method and presumably also by that of Folin and Wu, is 118 mgm. per 100 cc. of blood with a standard deviation of about ± 22 mgm.

3. When rabbits are fed 1 gram of glucose per kgm. of body weight, there is a sharp rise in the concentration of sugar in the blood which reaches a maximum at about ½ hour after feeding, falls to normal at the end of 2 hours, and is frequently followed by a distinct hypoglycemia 3 hours after ingestion of the glucose.

4. When 2 or 4 grams per kgm. are fed, there is a tendency to form a plateau at the maximum which is more marked and is maintained for a longer time with the larger amount and hypoglycemia is not apparent during the 3-hour period studied.

5. The rate of descent is more rapid the higher the concentration of sugar in the blood, after it has once begun, though heavy doses will delay its onset.

6. There seems to be a maximum rate of absorption from the intestine beyond which the organism can not go, regardless of the amount of sugar fed.

7. Because of the rather striking variations in the characteristics of the curves obtained after feeding different amounts of sugar, it is suggested that the standard dosage of glucose be adjusted to the body weight in sugar tolerance tests.

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The phase rule, le Chatelier's theorem, and the other principles of physical chemistry may be said to represent the physiology of chemistry, inasmuch as they deal with the deportment of systems, as opposed to the older morphological or structural point of view of the earlier chemists.

The present-day conceptions of the phrase rule and of the theorem of le Chatelier may be given in the form in which one finds them in a modern text on physical chemistry. "The Phase Rule states that, a system consisting of n components and r phases is capable of $(n-r+2)$ independent variations. That is, the number of independently variable quantities or "degrees of freedom" of such a system is $(n-r+2)$. This refers, of course, to the equilibrium state finally reached. Denoting the number of degrees of freedom by f, we can write the Phase Rule in the form of the equation—

$$f = n - r + 2.$$

"The principle (of le Chatelier) may be stated as follows: When a factor determining the equilibrium of the system is altered, the system tends to change in such a way as to oppose and partially annul the alteration in the factor. The same idea is conveyed by saying that every system in equilibrium is conservative, or tends to remain unchanged. That is, considering a physical or chemical system in equilibrium, the equilibrium being fixed by the nature of the system and conditions such as temperature and pressure, the principle states that if we alter one of these conditions or parameters, say the temperature, the system will change in such a direction as to tend to annul this change in temperature."[1]

If the reactions in living matter are physico-chemical reactions, the principles of physical chemistry should apply to some phases of physiology. And if physiology has any contributions to make to evolution, these contributions must be in the applications of its fundamental principles.

Various definitions of physiology have been given, one of which is that physiology is the application of physics and chemistry to the processes in living matter. Another definition given by Burdon Sanderson (1893) is "physiology is the study of the relations of the parts of the organism to each other." Burdon Sanderson pointed out also that the processes in the organism go on in the interest of the organism.

More recently Haldane (1914) has paraphrased this remark by saying that the changes in the organism occur in response to changes in the environment in such a direction as to conduce to the continued life of the organism. I have elsewhere (Hastings, Coombs and Pike, 1921) expressed the view that certain phases of adaptation may be considered as a special case of the theorem of le Chatelier. I have also (1915, 1921) given two specific examples under this theorem, that of the dog when the temperature of the environment is raised, and that of the cells of the respiratory center when a change occurs in the volume of blood flowing through them in unit time. In each case the reaction of the organism or of the cells is opposite in direction to that of the change occurring in the environment.

[1] Lewis, A System of Physical Chemistry, Vol. II, p. 229, p. 109.

tion is as yet but little understood, by which the reaction of a dog to a change in the external temperature occurs, or the nature of the respiratory system, to see that the mechanisms by which these reactions are brought about in higher organisms have most certainly arisen in organic evolution. Despite the arguments of those who look only to the physico-chemical phases of the life processes in organisms, I see no good reason why the physiologists should, at present, relinquish the idea of adaptation in the Darwinian sense; and if I am right in my contention, it is these reactions which lie at the bottom of all adjustments of the organism to the environment. By the same token, it is these reactions which should be of great interest, from the point of view of physiology at least, in the whole study of ecology (Pike, '23).

The biologist may again recall that the origin of the term ecology is to be traced back to Treviranus. Among recent reaffirmations of the principle of adaptation, one may cite that of Hooker (1917). In his consideration of Liebig's "Law of the Minimum," Hooker points out that the cases in which it does not hold—*i.e.*, the cases in which the growth of the plants is greater than would be expected from the amount of the particular inorganic salt which is present in least concentration—are of the greatest interest, and he cites these as examples of adaptation.

The foregoing statements serve to point a moral concerning some present-day tendencies in biology and more particularly in physiology. The attempt at the rigid application of the principles of physical chemistry to processes in the organism without reference to the biological, but not vitalistic, elements may lead us into error. It is worth pointing out, too, that the phase rule as such is not directly applicable to the processes in living organisms. The reason for this is not far to seek.

The conditions of equilibrium in slow reactions are considered in detail under the phase rule of Willard Gibbs. It is a primal requirement for the establishment of equilibrium in any system that the position or point of equilibrium may be approached from either direction. The processes in living organisms are, in part at least, not reversible reactions, but reactions which go on in one direction only. One can not deny, of course, that there are many reversible reactions going on in the organism. Compounds may be built up in a muscle, for example, which pass through reactions which are in part reversible. The glycogen of a muscle is broken down to lactic acid in muscular contraction, and the greater part of this lactic acid may be resynthetized to glycogen in the period following contraction. The remainder of the lactic acid is reduced to carbon dioxide and water. The animal organism does not, however, resynthetize the carbon dioxide and water to a carbohydrate, that process being reserved for green plants alone. While certain particular reactions in metabolism are reversible, the process as a whole is not reversible. Although Cuvier (M. Henderson, 1913) probably had little knowledge of reversible and irreversible reactions, he recognized clearly that

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THE FUNCTION OF THE VESTIBULAR APPARATUS

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The vestibule, as distinguished from the cochlear or auditory portion of the internal ear, is a circumscribed peripheral receptor present in all vertebrates from the Cyclostomes of the middle Ordovician period up to the present. It includes the saccules, lagena, utriculus, ampullæ and semicircular canals with their appendages. It does change somewhat with the type of animal chosen for study, the saccular and canal portion being represented in the Cyclostome Myxine or Bdellostoma by one canal with two ampullæ and by two canals in Petromyzon. In the Selachostomes and Pneumones (cod reptiles of the lower Silurian) (Dohrn and Ayers), it is much larger than in any extant form, the dimensions of the bodies being in centimeters rather than in millimeters or their fractions, as is the case in present day vertebrates. The general anatomy of the vestibule is given in the papers of Retzius (1), Ayers (2), Bigelow (3), Dohrn (4) and others. Although a few plates give the vestibular supply of the vestibule of the lost pig in detail.

Hensen (5) has given four criteria of a receptor, namely, the anatomical, the physical, the physiological and the psychological. The case for the vestibule may be examined briefly with reference to the application of these four criteria.

Anatomically, as well as physiologically, the vestibule belongs to Sherrington's group of proprioceptors. It is protected from direct contact with any ordinary agencies, or from slight agitation by any ordinary changes in the external environment. The agencies which stimulate its receptive organs and sensual elements must ordinarily arise within the body itself. The general structure of the vestibule suggests that the fluid contained within the labyrinth cavities and the otoliths in the saccule and utricle may act upon its specific nerve endings through the medium of a mechanical pressure generated by the inertia of the fluid or otoliths when the head is moved in any of the various planes of space.

Regarded from the point of view of physics the idea that mechanical pressure may be the appropriate stimulus to suppose not

only by theoretical considerations (7), (8), (9) but by actual experiment as well.

Physiologically, excitation of the vestibule gives rise to certain objective reactions of the eyes, the muscles of the limbs and trunk, and of certain viscera. Not all of these reactions are peculiar to excitation of the vestibule, but some combinations of reactions can be found which do appear to be peculiar to excitation of the vestibule.

Considered psychologically, the view recently taken by psychologists is that there is no mental quality arising from excitation of the vestibule that is discoverable on introspection. The general mental picture arising from any of the various experimental methods of excitation of the vestibule is rather to be considered as the integration of the various organic results of vestibular excitation and the mental consequences of these organic results than as something due to the vestibular endings alone (10).

The literature on the vestibule is enormous, probably including in all its phases about two thousand separate papers. A complete bibliography alone would require more space than can be devoted to the entire paper. The most complete recent bibliography of the subject is given by Griffith (11). Only a part of the physiological reactions arising from excitation of the vestibule can be considered at this time, without going very far into the vexed question of its central connections in the nervous system.

Although the early work on the vestibule was done on birds and mammals and even on the human subject, it seems best to depart from the traditional historical method of presentation and consider the effects of vestibular excitation or extirpation with reference to its comparative relationships. Although no true phylogeny of function can be given until the paleontologist and systematist have given us a more complete and logical account of vertebrate ancestry and descent than is now available, there is still a sufficient amount of difference in the reactions to vestibular excitation at various levels in the animal phylum to make such a method of presentation worth while.

The vestibule has been generally considered to be related to the maintenance of equilibrium. It is evident, however, that it cannot be the main receptive organ for the maintenance of equilibrium in invertebrates, since they do not possess it. A general account of the mechanisms in invertebrates has been given by Bethe (12), who points out that some of them maintain their normal position in space merely by their structure, that is, the distribution of the mass of the body with

reference to its center of gravity. Some dead organisms will float in water in the same position as the living organisms, and their position will be reversed when put into a fluid of a different specific gravity. Other invertebrates have so-called auditory pits containing grains of sand or other solid matter. Kreidl (13) washed the sand out of the auditory pit of a crustacean and replaced it with iron filings. The animal's movements could then be controlled by holding a magnet near it in the water. A considerable number of invertebrates have well developed otocysts, removal of which entails severe disturbances in locomotion. The literature is given by Frohlich (14), Prentiss (15) and others.

Vestibulum in vertebrates. Passing over the long and unfilled gap between vertebrates and invertebrates, and even over the lower forms of what some consider vertebrates, we may take Amphioxus as the next type.

Amphioxus. Willey (16), in describing this form, states that "Besides lacking differentiated lateral fins, Amphioxus differs fundamentally from the higher Vertebrates in the absence of a cranium, of paired eyes and paired or unpaired auditory organs" (p. 47).

"Amphioxus exhibits the characteristic vertebrate bilateral compression of the body in a very typical manner. It is obvious that such a shape of body is highly unfavorable for the maintenance of the equilibrium except with the assistance of some special mechanical and sensory apparatus. Now in Amphioxus the metapleural folds, whatever their exact function may be, do not serve in any way as balancing organs; and, as mentioned in the text, Amphioxus has no means of maintaining its equilibrium when not actually swimming" (p. 43–44).

It is perhaps because of the difficulty of maintaining a body of such form in equilibrium that the locomotion of Amphioxus is so irregular, —"a rapid, curiously irregular wriggle"—periods of swimming forward, sometimes upon the back, sometimes upon the abdomen in the position of ordinary fishes (17) being interrupted by somersault movements and backward swimming (18) until the animal settles down and burrows tail first into the sand (19).

Cyclostomes. Experimental removal of the vestibule of Cyclostomes has been done successfully but once. Ayers (2) removed the vestibule from Bdellostoma dombeyi and found no appreciable disturbances of locomotion. Some of the same considerations which apply to Amphioxus apply also to Cyclostomes. Although there is a somewhat rudimentary vestibule—whether primitive or degenerative does not make so much difference from our present point of view—the locomotion of

Cyclostomes does not partake of the character of the strong swimming fishes, and the whole motor mechanism—nervous and muscular, shows a less high stage of development. Like Amphioxus, Bdellostoma has no fins, and its body is more like the cylindrical Balanoglossus than like the bilaterally compressed Amphioxus. While it has the sense organs for the perception of the change of aspect of the head in space, it has no paired effectors or fins which may react in a special way to excitation of the vestibule. As Willey points out, the maintenance of equilibrium is easier when the body is cylindrical than when it is bilaterally compressed, as in Amphioxus. Bdellostoma swims with eel-like movements of the body, and when at rest on the bottom, curves some part of the body, usually the tail, to one side or the other to keep it from rolling over.

Selachians. The relatively large size of the vestibule, and the fact that the soft cartilage in which it is embedded makes operative approach easy, have been among the reasons why the Selachians or Elasmobranch fishes have been favorite subjects of experimentation. The methods of experiment have varied from complete removal of one or both vestibules, section of the eighth cranial nerve, removal of the canals and ampullae without injury to the utricle, removal of the utricle without injury to the ampullae, exposure and mechanical excitation of the otolith of the utricle, and testing the reactions of the fins and eyes to a change in position of the head when the vestibule is intact. Most of the observations have been made upon some genus of the dogfish and upon rays. All three canals and the utricle and the saccule are well developed in Selachians, and we have for the first time in the vertebrate phylum, a fully developed vestibule with all its parts as it exists in higher vertebrates. Paired fins are also present in Selachians, and we have again the first complete mechanism for the maintenance of the equilibrium of a bilaterally compressed vertebrate body—sensory mechanism, central nervous system and effectors which may act as balancing organs. The maintenance of the normal posture of fish requires some mechanism, as Manoyer (20) showed that fish swim in labile rather than stable equilibrium.

Such a balancing mechanism—a receptor, a central system and the effectors—fulfills Goltz's (21) conditions, and, although found for the first time in living vertebrates in the Selachians, exists in a modified but physiologically adequate form in such free-swimming invertebrates as the crayfish and the lobster. But it is well to state here that the vestibule does not constitute the only receptor which is concerned with

the compensatory movements of the eyes persisted after such removal of the ampullae, except those following rotation of the animal about its dorsoventral axis, i.e., in the plane of the horizontal canals. It seems clear, from Maxwell's results, that the otoliths are concerned, along with the ampullae, in the perception of angular acceleration.

The converse of this state of affairs,—reaction of the ampullae after complete removal of the utricular otoliths—was also tested out. When the utricular otolith is removed without injury to the ampullae, all the compensatory movements of the eyes in response to rotation of the animal about any one of the axes of the body persist.

After removal of the ampullae, with conservation of the otoliths, the compensatory movements of the eyes, although prompt, are noticeably slower than when the vestibule is intact. If seized while in the water, the animal strongly resists the attempt to turn it back downward; but one feels that this resistance is neither as prompt nor as strong as in the intact animal (27). In swimming there is a tendency to sway from side to side about the longitudinal axis like a boat insufficiently ballasted. When the otoliths alone are removed, with conservation of the ampullae, the compensatory movements are slightly slower than in the intact animal. Swimming is apt to be accompanied by a slight rocking movement from side to side; and if turned back downward in the water, the animal rights itself promptly, but may turn almost or completely over before coming to rest in the normal upright position.

The otolith of the utricle is more easily accessible in the shovel-nosed ray (Rhinobatus) than in the dogfish. It may be exposed and stimulated mechanically in situ, after removal of all the ampullae. Maxwell (28) found that "pressure on the right side of the otolith of either ear produces the same eye movement which results from rotation of the body to the left about its longitudinal axis," i.e., the right eye is depressed and the left rolled upward, "and that pressure on the anterior side of the otolith produces the same movement of the eyes which results from tilting the head upward,"- the eyes are rotated forward about their visual axes. It is the displacement of the otolith and not the weight due to the otolith which is the actual stimulus, and it is the direction of the displacement which determines the direction of the compensatory movement.

Maxwell's conclusions are that the assumption of a sharp differentiation of function between the otolith-bearing portions of the otic labyrinth and the semicircular canal portion is not justified by the facts.

The two parts of the labyrinth reinforce each other, for the reactions produced by either one alone are slower and less vigorous than when both sets of organs are intact. The two parts may not have identical functions, however, since rotation in a horizontal plane is completely lost after removal of the semicircular canals.

Removal of all semicircular canal and vestibule of both auricles has the same effect as complete destruction of the vestibules or section of the eighth cranial nerves.

Some investigators have reported negative results from complete bilateral or unilateral destruction of the vestibule, but more recent work tends to show that there is a definite train of symptoms following such a lesion. All compensatory movements of the eyes and fins to rotation of the animal about any axis of its body are completely and permanently lost after bilateral lesions. The animal swims indifferently in the back upward or belly upward position. A weak animal comes to rest on the bottom of the tank on its side or back, but a vigorous animal will right itself by reacting the bottom, the reaction being due, in Maxwell's opinion, to the sense of touch. As he expresses it, the geotropic responses of the animal are lost, but the stereotropic responses are retained. Some degree of recovery may occur, but there is always some disturbance when the fish is made to swim rapidly, as a rule.

The effects of unilateral lesion are less severe. The eye of the injured side turns downward and the eye of the sound side turns upward 30° to 40°. The body is turned markedly to the injured side. In swimming, the fish may roll to the injured side. Normal swimming seems possible, but disturbances of locomotion, particularly when the eyes are closed, are very apt to appear. The displacement of the eyes is persistent as long as the animal lives, two weeks in some cases. Compensatory movements of the fins and eyes are still elicitable on rotation in any one of the three planes of space, although often weakened and difficult to observe.

Considerable discussion has arisen as to whether the effects of vestibular lesions are due solely to the loss of a peripheral receptor or due largely to the irritative effects of the injury. Gaglio (28), Konig (29) and Breuer (31), the last using pigeons, have applied cocaine to the vestibule. Gaglio observed, in the Mediterranean dogfish, the same results after the application of cocaine as after anatomical removal of the vestibule. This strongly indicates that irritative processes play but little part in the effect of vestibular lesions in this form. Again

one would expect about as much irritation from destruction of the ampullae together with their specialized nerve endings as from complete destruction of the vestibule. Yet Maxwell found that the reactions of the animal, after removal of the ampullae alone, differed from those of the normal animal only slightly. Whatever effects of irritation may have been manifested elsewhere, there were extremely few which were apparent in those reactions which we have come to look upon as characteristic of vestibular excitation. It is my opinion that the effects of irritation in connection with vestibular lesions have been greatly over-emphasized. One might make the same statement with reference to many other lesions of receptors or parts of the central nervous system.

One other permanent effect of bilateral vestibular lesions was brought out by Gaglio, and that is the loss of muscular strength following this destruction. Parker (32) also remarks that the muscle tonus of the animal is noticeably reduced after bilateral lesions. This can scarcely be due to any effect on the muscles themselves, but rather to the loss of afferent impulses normally concerned in the maintenance of tonus or the utilization of muscular strength.

Both Parker and Maxwell agree that the saccular otolith has little or nothing to do with vestibular function proper. Parker regards it as a part of the mechanism of hearing in fish.

Although Mach showed the physical improbability, even impossibility of actual currents of fluid in the semicircular canals, the hypothesis of stimulation of the ampullar nerve endings by currents set up in the canals has been very popular among otologists. Maxwell freed the horizontal canal for nearly its whole length, ligated it near its posterior end and, after cutting it, raised it so that its plane was vertical. Rotation of the animal in a horizontal plane, about the axis of the canal in its new position, was attended by the usual reaction to rotation. No possible currents could have been set up in the canal under these conditions, and it is difficult to see how any pressure could originate in the canal to be communicated to the ampulla. In the statement of the mechanism of excitation of the ampullar endings, Mach, Breuer and Crum Brown supposed that pressure arising from the lag of the fluid in the canals during rotation was transmitted to the ampullae. Implicitly, it was assumed that the pressure arising in the utricle from the inertia of its contained fluid would either not be transmitted to the ampullae or, if so transmitted, would not be effective in stimulating the nerve endings. When one considers the mechanics

fact that bony fishes may, in time, recover almost completely from lesions of the internal ear. The immediate effects are therefore due to shock, and not to the loss of a particular receptor.

Parker (34) found that Fundulus heteroclitus, after section of both eighth nerves, lost the power of maintaining equilibrium when swimming rapidly, and swam in any position in spirals or even in circles. In his discussion of Tullberg's view, Parker (p. 201) admits that Fundulus may recover fairly well in a few days after the operation, so long as it swims slowly. But it invariably loses this power of maintaining equilibrium if made to swim rapidly in a large amount of water. The greatest length of life of any animal after operation was six weeks, but the effects were clearly observable throughout this period. Parker attributed the partial recovery of the animal and its facility in swimming slowly to increased use of the eyes. He admits that this is an assumption on his part, but there is much evidence drawn from the study of reactions in invertebrates to support such a view. Until this assumption is definitely shown to be untenable for bony fishes, Parker considers that Tullberg's conclusion is premature.

My own view is in accord with Parker's. The same arguments adduced against peripheral irritation and shock in the case of vestibular lesions in the cartilaginous fishes apply with equal force in the case of the bony fishes. It may be shown that the loss of any one sense does not entail any very severe permanent disturbances of locomotion in higher animals. One might conclude, therefore, that no one of these sensory mechanisms had much to do with locomotion. The argument should be carried a little further, however. If no one sensory mechanism has much to do with locomotion, it should be possible to eliminate all of them and still retain relatively unimpaired powers of locomotion. For if the whole is equal to the sum of all its parts, the whole effect should be relatively slight. Common experience points very strongly to the conclusion that the total effect is not relatively slight. Since Euclid's argument has now been subjected to criticism and analysis for some centuries without suffering serious damage, I am inclined to suspect that the fault may lie in some of our common assumptions concerning the functions of various sense organs rather than with Euclid's reasoning. The presumption is strong that some of the quantities concerned in physiological reasoning, e.g., the effects of removal of various sense organs, are really variables instead of constants, as Tullberg's line of reasoning assumes. The reason for this variation lies less in some undefined shock effect than in the compensation for the loss of one sensory mechanism by another.

Bigelow's observations on Carassius are in agreement with Parker's. The facility of compensation in equilibrium when swimming slowly after loss of both external ears was lost when the fish was made to swim rapidly in a large body of water. The effects are permanent.

Parker (25) found that the rotary ataxia suffered loss of muscular tonus and the loss of the power of maintaining equilibrium after section of both eighth cranial nerves. Most of the loss of such tonus is spent in lying on the bottom of the tank, often with the ventral side up. When they swim it is usually in a slow rolling way, and when driven to rapid motion, they swim in grotesque spirals.

Bethe (2) and previously found much the same effects in Carcinus. Section and destruction cerebrospinalwise after vestibular lesions. After a unilateral lesion, Bethe observed a nystagmoid effect on the injured side. There is no loss of synchronism of the two sides in respiration, but the gill stroke on the injured side are not raised as high as those on the uninjured side.

Ewald (26) confirmed all the old. Ziegelin (39) also the loss of strength noted by Ziegler in the frog. There were also severe disturbances of equilibrium.

Retterhoss histological conditions in the batrachian ear preclude such exact and detailed study of individual parts of the vestibule as is possible in the elasmobranch, on the other hand, the development of limbs makes possible the observation of the effects of vestibular lesions upon animals which have reached the second stage in the development of the vertebrate locomotor apparatus. There is also a further development of the skilled nervous system. By means of the limbs, these forms maintain a fixed position when at rest on the surface of some solid object, and hence are the first vertebrates which may be tested on the turning table in such a way as to show the effect of vestibular excitation upon the resting animals. The batrachians include also forms which, although they retain their gills throughout life and live in the water, show their homologies to lower orders forms which retain their gills during the early part of life only and then emerge from the water upon the land, and still other forms which become so specialized that, after undergoing metamorphosis, their mode of progression upon the land is by hopping or jumping rather than by walking or crawling.

Shumway (37) used a pseudobranchiate form Necturus (Menobranchus) for experiment. He found that destruction of the internal ear on one side was followed by rotary movements of rotation of the body,

always to the injured side, about its longitudinal axis. The movements are apparently involuntary and without fatigue. If for any reason these movements cease, they can be started again by excitation of the animal.

Ewald (38) found that in Salamandra maculosa, there are only slight motor symptoms as a result of vestibular lesions. After a unilateral lesion the legs of the opposite (uninjured) side are raised abnormally high, while those of the same side may be dragged along the ground.

Laudenbach (39) removed the entire vestibule, unilaterally and bilaterally, and the otoliths alone in Siredon piscifornis. After unilateral lesions of the whole vestibule, the animal rotates rapidly about its longitudinal axis when swimming, turning always toward the injured side. At first it can not go forward at all without undergoing this rotation. The disturbances are more marked in young animals than in old. Adults recover sufficiently in three or four days to swim slowly without turning over, but not to swim rapidly.

Whether bilateral operation was done on the same day, or whether the second labyrinth was removed after an interval, severe and permanent disturbances of orientation followed, when the animal was in the water. The animal maintained its position on the ground or at the bottom of the tank well. It could not maintain its normal position at the surface of the water, but floated belly upward with the nose under water. Any recovery that may have occurred after removal of one vestibule was lost after subsequent lesion of the second, so far as deportment in the water was concerned, and little recovery was seen even after fifteen months. The difficulty in swimming was so great that the animal could not get to the surface for air, and the gills again began to grow if the animal was kept permanently in the aquarium.

No noticeable disturbances of movement or equilibrium follow unilateral or bilateral removal of the otoliths without injury to the ampullae.

The effect of unilateral and bilateral lesions of the whole vestibule in the frog is so well known from the work of Schrader (40), Ewald and others as to require no special comment here. There is torsion of the head and body to the injured side after unilateral operation, swimming in a circle to the injured side and turning to this side in jumping. The fore limb of the sound side is strongly extended as if the animal were bracing itself by it, while the fore limb of the injured side is strongly flexed.

Henry (31) found that tissues of the head partially or almost completely disappears after an interval of six weeks or so. If denervation is done at this time, the original symptoms reappear on all their original intensity, and no further amelioration of the conditions is observed so long as the animals live. If denervation is first and the vestibular operation afterward, there is no improvement in the condition of the animal as long as it lives.

Shambaugh (32) observations on the effect of unilateral or bilateral removal of the auditory pit and acoustic ganglion in the tadpole are of considerable interest in relation to the autopsy of vestibular functions. The fact that it is possible to produce at will practically a congenital absence of the auditory vesicle serves as a control for the experiments on adult animals, and has a direct advantage in the permanence, completeness and lack of injury to contiguous structures over operations on adult forms. And since it may be presumed that the various organs possess their greatest degree of adaptability during the formative period, such embryonic interference affords a most complete test of the power of functional adaptation for loss of a specialized receptor.

The larval frog passes through three stages in acquiring the faculty for swim, which Shambaugh names as follows: 1. The stage of immobility—the first thirty-hours. 2. the stage of spinal reflexes—fourth to sixth days; 3. the stage of maintenance of equilibrium, sixth day to maturity. At the close of the ten to twenty-six stage, the structure which is to form the future auditory vesicle is visible and may be dissected out. The auditory vesicle never regenerates after its complete destruction, including the auditory ganglion.

The larva makes no effort to swim in the first twenty-four hours, and it is only on the fourth day after the operation (about the sixth of larval life) that any significant difference in behavior is noticeable as a result of the lesion. At this time, when they, as well as the normals, are stirred up from their resting position on the bottom or against the sides of the dish, the operated individuals seem to quiver or wobble. There is a tendency to swim with the injured side lowest, and the rolling movements which occur about the longitudinal axis are always directed toward the operated side. On reaching the bottom they are able to right their bodies as the normals do. The characteristic disturbances in swimming become more marked in the next two days, but on the seventh day after operation many of the operated larvae may swim in a fairly direct course. On turning them, the rolling

movements reappear. There is improvement from this time on, but the larvae always incline more or less to the injured side, and momentary loss of equilibrium is noticeable whenever they become excited.

Metamorphosis occurred at the end of the third month. No difference in attitude, whether at rest or in motion, could be made out in the operated as compared with the normal individuals after metamorphosis. Streeter thinks that the disappearance of the symptoms does not indicate any cure of the condition set up by the lesion, but that a slight defect is more difficult to recognize in the frog than in the tadpole. As a corollary, he points out that the maintenance of equilibrium in the tail-swimming tadpole is dependent upon a more delicately balanced mechanism than in the leg-swimming frog. It might be pointed out, also, that a more perfect set of peripheral effectors for the maintenance of equilibrium is present in the frog.

After bilateral operation, there was practically no difference to be made out in the appearance of the operated and the control larvae for the first three days. On the fourth day after the operation, they are decidedly less active than the controls, or than larvae with one auditory vesicle only removed. They make no swimming movements, but wriggle along on the bottom of the dish. Seven days after operation, they are smaller than one-sided or control specimens. They are symmetrical in form, but are less active and make only unsuccessful attempts at swimming. Twelve days after operation, any attempts at swimming resulted in a series of somersaults, after which they sank to the bottom in almost any position. There is great difficulty in feeding, and wriggling movements along the bottom are not as well executed as on the fourth and fifth days after operation. The larvae could not be carried much beyond two months of life, as they seemed unable to get food, and to go to the surface for air as normal larvae do. They were only about one third as large as the controls at this time, and had no more ability to swim than they had a few days after the operation.

One may remark that the severity of the effects of a bilateral lesion is much more than twice that of the effects of a unilateral lesion. This great difference in severity of effects seems difficult to account for on any other basis than the compensation for the loss of only one auditory vesicle by the other. And the larval form, while it compensates quite as perfectly as the adult for the loss of but one ear, is much less able to compensate for the loss of both than is the adult.

Lombard found no noticeable defects after removal of the otoliths only, with preservation of the ampullae in the frog. Ash (02), however, reexamined the question. He calls attention to Breuer's (00) analysis of the spatial relationships of the otoliths in the pars of the frog, and the general parallelism of each of these to the plane of semicircular canal. He confirmed Lombard's observations so far as any effects upon the attitude of the body or the usual movements of progression were concerned. Ash brought out, however, three effects of the bilateral removal of the otoliths. There are three reflexes—the "righting" reflex, in which the frog assumes a position so that its back is upward, the ground supported only upon the tip of its nose and its hind feet; the stretching reflex, which differs from the ordinary crouch reflex; and the lid reflex of the eye. The first two are difficult or impossible of elicitation in normal frogs, while the lid reflex is usually present. After bilateral operation the first two, the "righting" reflex and the stretching reflex, were much more easily elicited than before, while the lid reflex failed in progressive limb movements.

After unilateral lesions of the otoliths the lid reflex is still obtainable at times. When the frog is turned up or down in a vertical direction, and particularly when it is moved downward, the lid reflex appears in the eye of the injured side. In lateral movement from side to side, as backward and forward in the same plane, both eyes, or one only, may close, whether the otoliths are intact, or absent on one side. Ash never observed closure of the eye on the normal side *only* after unilateral operations. If only one eye closes when the otoliths of one side are absent, it is the eye opposite to that of the ruined labyrinth. The relationship appears to be a crossed one. After bilateral removal of the otoliths the lid reflex persists practically undiminished; or when the frog is rotated in a horizontal plane about its dorsoventral axis it appears at decreased speed of progression only or is from side to side about its longitudinal axis. Under conditions of rotation, the lid reflex must be due to excitation of the ampullae. He considers the failure of the lid reflex in progressive limb movements after bilateral removal of the otoliths as the most characteristic effect. He attempts to show that Breuer's idea of the prevention of linear acceleration by the otoliths only is correct.

Graham Brown (08) investigated the effects of physiological excitations of the vestibule upon the respiratory movements of the frog. The effects of vestibular excitation are not peculiar, but are very similar to the effects arising from pinching the toes of one foot, and

apparently may all be comprised in the usual cycle of acceleration and augmentation of the respiratory movements. Bilateral removal of the otoliths does not affect the response to movement in a vertical direction, that is, to linear acceleration, but complete removal of both vestibules abolishes the response. Brown supposes that the ampullae may respond to movement in a straight line, and departs from Breuer's and Ach's views. Extirpation of both vestibules has no permanent effect upon the respiratory movements, although there is indication of some temporary loss of tonus of the muscles of the floor of the mouth.

Reptiles. The reptilian brain represents the highest type of generalized brain. The superficial layer of gray matter on the cerebral cortex has increased in amount, as compared with the batrachian brain (45) and there is some representation of the central terminations of the optic fibers in the cerebral hemispheres (46). In higher vertebrate phyla, there is development of the avian brain on the one hand characterized by the relative preponderance of the cerebellum, and the mammalian brain on the other hand, characterized by the relative preponderance of the cerebral hemispheres. The vestibule is enclosed in hard bone, so that sharply localized lesions of its various parts are not easy to effect, and the mode of reproduction renders the extirpation or transplantation of the embryonic vestibule more difficult than in some batrachians. The variations in body form, from the extremely elongated serpents without any limbs whatsoever, to the turtles with their extremely stable base of support for the body and long mobile neck, afford an opportunity for study of the effects of lesions of one peripheral sense organ upon various types of locomotion in one phylum, and upon various parts of the body as well.

Trendelenburg and Kühn (47) used the European swamp tortoise, Emys lutaria, for experiment. After unilateral operation, they observed torsion of the head to the injured side when the head was in the shell and bending of the head to the injured side when it was protruded. Slow progression on the land was not attended by any unusual disturbance. The torsion of the head and neck is permanent, but there is no turning to one side in progression. The swamp tortoise is more active in the water than on the land, and more marked disorders appear in swimming than in crawling. When at rest, the injured side is lower in the water, and in swimming there is movement in a circle to the injured side. Frequently the animal spins about a dorsoventral axis. It rights itself when placed back downward in the water.

Compensatory movements of the head are direct and are accompanied more rapid in the optic than in the labyrinthine. The loss of the vestibule does not alter the reaction of the head to a change of position. If the visual field is kept stationary, both the initial and the post-rotatory effects are obtained if the rotation is toward the injured side, but the after effect ceases if the rotation is toward the sound side. There is a point of some interest as it indicates that the vestibule has lost nothing of its property in relation to other directions in space since there is loss in the horizontal plane. If the visual field moves with the animal, the rotation effect fails when the rotation is toward the wound side.

Bilateral lesions are not attended by any unusual manifestations when the animal is at rest. The movements of head are slow, but just otherwise abnormal. The head moves back and forth without resistance when the animal is shaken. If right-hand action placed back downward on the ground, but may remain in the back downward position when swimming. It turns to one side or the other and may even turn in a circle when swimming.

The diagram (the last given) the most below an exhaustive account of the vestibular symptoms in rabbits. The new observations that have been along the lines of the unilateral and bilateral extirpation of the vestibule and observed stimulation of the ear. The results on extirpation are in general agreement with those of Ewald-Bethe and Kubo. Along with the tension of the head following unilateral operation there is a displacement of the nose such that the axis of the injured side is turned upward and the other downward. When resting at rest, the limbs of the injured side are flexed close to the body while those of the opposite side are extended. Food is grasped incorrectly and promptly after the first few days. The tension of the head lasts as long as the rabbit lives, right corrects in the case of some operations. Any diseases in the months occurring in the first months of recovery is lost after decerebration. The lateral tilt becomes even more marked than before.

The effects of bilateral operations are more severe. The animal floats head in the water, and rabbits will float on its back without righting itself. Occasionally because of the peculiar shape of its body, will not remain on its back in the water at all. In swimming, Rabbits may go in a small circle maintaining on its side with the back directed toward the center or it may turn about its dorsoventral axis and stop with all limbs straight extended and its nose pointing almost straight upward.

Control specimens grasped pieces of fish placed in the enclosure readily and accurately. The body is held immovable and the neck and head are suddenly thrust out. The jaws close with a snap as the food is reached, and the head is quickly retracted. I have seen a turtle, Chrysemys, repeatedly grasp at food after bilateral operation, without ever touching it. The head was retracted after each failure and again thrust out, only to go to one side or the other or above or below the desired morsel. Although evidently hungry, the animal might finally give up the attempt to grasp the food and crawl away. The broad flat body of the turtle affords a secure basis of support, and the inability to grasp the food signifies that the animal is unable, even with the aid of its eyes and muscular sense, to control the rapid movements of the head accurately. These erratic movements of the head bring out sharply the importance of Crum Brown's statement that the function of the vestibule is to give information of the aspect or change of aspect of the head in space. The vestibule is a proprioceptive structure whose biologically adequate stimulus arises from the aspect or change of aspect of the head in space, from the aspect or change of aspect of no other part of the body in space. Its loss leads to centripetal ataxia.

Electrical stimulation of the ear of the turtle is easily accomplished (50). When the direct current from fifteen to twenty dry cells is passed through the head from one ear to the other, there is a movement of the body, brought about largely by the hind feet, so that the head is turned toward the side of the anode. The response persists after decerebration.

Loeb (51) brought out the important principle in the horned toad Phrynosoma, of the algebraic summation of optical and vestibular stimuli or, as he calls them, heliotropic and geotropic effects in the elicitation of compensatory movements during and after rotation. When the lizard is rotated, with ears intact and eyes closed, a speed may be found which will elicit barely perceptible compensatory movements of the head during rotation. But on suddenly stopping the rotation the animal will exhibit very marked compensatory movements. With the eyes open the effects are just the reverse. Two animals, one with the eyes open and the other with the eyes closed, may be placed on the same turning table at the same time, and these differences in deportment noted under exactly the same conditions of speed of rotation and suddenness of stopping. If the visual field is rotated at the same speed as the animal, the effects with the eyes open are the same as with the eyes closed. If an endless roll of paper with wide vertical lines marked

on it is retained when the animal. Even the compensatory movements of the head during the movements of the paws, but soon after the paws has ceased to move.

The fact that the effect of rotation may persist in animals—dog (Ewald), turtle (Trendelenburg and Kalin) after removal labyrinth of the vestibule when the eyes are open but not when the eyes are closed had been known for some time previously; and the neglect of precautionary measures has led to many incorrect conclusions. The fact that, in Pleurodeles, the most marked compensatory movements of the head appear after rotation has ceased when the eyes are closed seems to disprove effectively all Goltz's old analogy of the heavily loaded wooden head and the view that the compensatory movements of the head during rotation arise from the inertia of the head itself. Grünberg (22) had concluded from experiments on the dog that the "optic" was the only constant element in the nystagmus of the vestibule during rotation; and Maxwell (34) showed on Pleurodeles that the labyrinth effect was the essential element. The two names appear to indicate exactly the same thing.

Trendelenburg and Kalin found that after bilateral lesions of the vestibule the common lizard Lacerta agilis showed no abnormal position of the head when at rest. Frequent tilting, however, swaying of the head from side to side would appear. These movements were especially noticeable after the animal had been making rather vigorous movements of the body and had come to rest. The animals had great difficulty in catching the mealworms on which they were fed, the head swaying about so much that it was largely a matter of chance when a worm was caught.

Hence this examination is of interest from the theoretical point of view to investigate the effects of vestibular lesions in serpents because of their peculiar method of progression. The effects of vestibular lesions in the adder are manifested in various ways. There is a general feebleness and sluggishness of movement. The snout snake raises its head slightly above the ground on crawling but, after operation, it rests or supports the lower jaw on the ground, and there is a tendency to turn to the injured side after unilateral operation. It held by the middle of the body, and on several attempts to raise its head to reach the outstretching hand, but, in so doing, it turns its head around in a circle and rotates about the long axis of its body. The head is lower on the injured side when crawling at rest. It may even rotate about its long axis by rotating. If placed on its back, it rights itself, turning always

to the injured side. Movements of the head may persist for some time after crawling has ceased. These observations were confirmed by Trendelenburg and Kühn. They found that the rolling movements of the body occur only when the snake is on a smooth surface, and not on a rough.

After bilateral operation, the position when at rest differs in no noticeable respect from the normal. But uncertainty is apparent as soon as the snake attempts to move. The head moves back and forth like a pendulum, with only momentary interruptions. If the snake is grasped by the anterior part of the body and shaken, the head moves aimlessly back and forth. In a normal animal the head moves along with the body. In swimming the head sways from side to side with the movements of the body instead of maintaining its median position, as in the normal specimen. Spiral bending and swimming occur at times. The back upward position in the water is very stable, even when the eyes are closed with collodion. Only when dropped from a height does the snake turn ventral side up in the water.

Most of the actual information derived from a experimental source as to the function of various parts of the vestibule and the mechanism of excitation has come from the study of the representatives of the three lower phyla of vertebrates. The rôle of the receptor in giving the animal control of the movements of the head is clear from a study of the experimental results. The difference in effect of unilateral or bilateral lesions upon various closely related forms with different methods of progression, but all of which are alike in possessing a central nervous system which does not show any very high degree of development, as compared with the mammalian type, and which vary from forms which spend their whole life in the water to those which enter the water only incidentally, are better shown here than in any other animal types used for experiment. One finds all variations from no receptor whose normal mechanism of excitation depends upon the aspect or change of aspect of the head in space, and no special effectors for correcting any variation of the head or body from the normal position, to a specialized receptor and a complete equipment of effectors. In between these extremes there are rudimentary or primitive effectors, or even their total absence. The eel and the serpent have no special effectors aside from the general musculature of the body. There are no paired appendages. But the presence of the specialized receptor enables the animal so to manipulate or control its general body musculature that all variations of the body and head from the normal position may be

regulated promptly and effectively. Similarly, lizards may suffer the loss of all four limbs but, as long as the vestibular endings are intact, the normal reflexes are free of marked control in the water. One need go no further than the forms already described to find ample reason for doubting Ayers' (1) conclusion as to why the vestibular mechanism should have been evolved.

Passing from these lower vertebrates with the generalized nervous systems to the birds and mammals, the nature of the problem changes somewhat. The increasing efficiency of the central nervous system as an integrating mechanism, and the increasing complexity, with a possible increase in the sensitivity, of a peripheral sensory mechanism shift the emphasis from the effects of the extirpation or lesions of one peripheral sensory structure as we find it in lower vertebrates to the ability of a more developed central integrating mechanism to compensate for and largely mask the effects of the loss of a single peripheral receptor. The development of the exteroceptors, and particularly of the distance receptors, in the land-living mammals and birds introduces another element. The free, powerful swimming fishes are dependent upon proprioceptive stimuli for the maintenance or control of the position of the body while in the water and not in contact with the bottom or other solid or motionless objects. There are few fixed points of reference in their environment of which they take cognizance through the exteroceptors, contact or distance. Probably there are few fixed points in the open ocean through or by which a shark orients itself, but with the usual land-dwelling mammals, and particularly man, the case is different. There are few times in the life of an ordinary mammal on land when it is not in contact with the earth or some other fixed object. In the water or in the air, the case may be a little different, and the study of the effects of vestibular lesions in birds and mammals should be carried out under conditions as similar as possible to those under which the vestibular mechanisms is of greatest assistance to the lower vertebrates, that is, when an animal is prevented from coming in contact with fixed objects in the environment, or when, through elimination of vision, it is prevented from orienting itself through the visual exteroceptors.

It is to be presumed that the effects of vestibular lesions in lower vertebrates are more nearly objective than subjective. In the human, subjective effects appear, such as vertigo and the general group of sensations which have been included under the term sensation of rotation or of apparent movement. There is presumably an organic basis for

such sensations, but their analysis leads one into the central nervous system rather than to mere lesions of peripheral receptors. A fundamental principle in the interpretation of the effects of central nervous lesions in different animal forms enters in here,—the principle of the migration of function toward the anterior or cephalic portion of the central nervous axis (46).

Birds. The vestibule of birds has, from the time of Flourens to the present, held a central place in the study of its function. The work of Flourens on the experimental elimination of the internal ear marked the first stage in the experimental study of this receptor. The second stage began when Goltz (21) formulated his theory that the semicircular canals were sense organs for the perception of the position of the head in space. He proposed a hydrostatic theory of stimulation of the vestibule through changes in hydrostatic pressure of the fluid with the changing position of the head in space. The hydrostatic theory fell into disuse after the development of the dynamic theory of Mach, Breuer and Crum Brown. There is abundant evidence that the dynamic theory is applicable under many conditions of animal activity, but to discard totally the hydrostatic theory at present seems premature. We have only to remember that the change in the position of the eyes of a normal dogfish lasts as long as the head is held in the changed position, long after any inertia of the fluid in the vestibule has been overcome, to see one possible application of the hydrostatic theory. That it is not necessarily due to displacement of the otoliths is shewn by the fact that the same displacement of the eye occurs after removal of the otoliths.

Ewald (56) called attention to the state of the vestibular problem at that time in the introduction to his first paper on the function of the vestibule in birds. Experiments up to that time had yielded, as they have up to the present, conflicting results. The effects of vestibular lesions in birds, up to that time, had been more severe and more permanent than the effects of similar lesions in fishes or mammals. The most reliable work on fishes has appeared since 1887, and the effects of vestibular lesions in birds have gained enormously in clearness, accuracy and fulness of detail from the work of Ewald himself. Better methods of operation have been devised in mammals, and the observations of the effects of lesions have been more carefully made since that time, but the differences in effect which Ewald noted are still more or less apparent. The immediate effects of operation are very similar in severity in all types of vertebrates which have come under my

observations, but the results always differ in intensity, and particularly under varying conditions. The effects of bilateral lesions in larval frogs are more severe than the effects of similar lesions in birds or mammals. The accuracy and permanency of the effects in frogs are fully equal to those in birds, and quite fully equal to the effects observed in mammals under ordinary conditions. Ewald's statement, therefore, needs more extension in view of the greater number of facts now available on a greater range of animal forms.

Ewald stated three possibilities, one or more of which might account for the difference in the effect of vestibular lesions: 1. There may be a difference in the topographical relations of the vestibule to different forms of animals so that contiguous structures may be damaged to different degrees in vestibular operations on various animals. For example, neighboring regions of the brain may be damaged more in birds than in mammals, and not at all in fishes. It was not until Lange (37) showed that the effects of vestibular lesions in birds were not dependent in any degree upon injury to the cerebellum that this question was answered for birds. And no good experimenter need inflict any injury whatsoever upon the central nervous system of any animal in operating upon the vestibule. 2. We may say that the vestibule has different functions, or at least different degrees of the same function, in widely separated groups of vertebrates. This may favor a rudimentary development of the vestibular organ, somewhat better development, and finally the highest development of all. By way of comment on this possibility, it may be said that we have no index of the acuity of the vestibular endings in any vertebrate except man. It is possible, of course, that the acuity of the vestibular organ, to follow Ewald's terminology, may vary in different animal forms just as the acuity and discipline of vision or the acuity of smell may vary, even in different individuals of the same species. 3. We may say that the vestibule has the same function in different animal forms, and that, following its destruction, the same functional defects appear in all of them; but these defects may be more or less partially compensated by the many conditions under which the animal lives. Under these many conditions are included the general symmetry of the body, the length of limbs, the length of the neck, the number and size of the points of support, the topography and size of the supporting surface, as relative to the form of the body and the medium in which the animal lives. These considerations are important. As I have mentioned above, the animals I use, the best are under all ordinary circum-

stances, in contact with a solid surface, that of the earth itself, only occasionally, depending largely upon habit, going into the water or into the air. Birds go into an even more unstable medium than water,—the air. It seems to me that we should now add at least one more consideration, 4, that of the degree of development of the exteroceptive sense organs, particularly the distance receptors, and the degree of development of the central nervous system itself.

In order to test out these various possibilities, Ewald made a series of experiments on various representatives of a single phylum and chose birds. In this phylum, if one does not choose genera too remote from each other, the topography of the vestibule is too similar to permit of many differences in effect arising from varying degrees of injury to contiguous structures, and one would think still less of a difference in function of the vestibule in different families of the same phylum. On the other hand, the static conditions in different families and genera of birds vary greatly according to whether they run, jump, fly or swim. The mode of station at rest also varies, as some lie, sit, stand on two legs or on one. Such a study should bring out the extent to which the functional disturbances following lesions of the vestibule are dependent upon the static conditions of the birds chosen for experiment. Ewald's results are given in the accompanying table.

Ewald emphasizes the fact that the difficulty in flying is more marked in birds whose wing movements are more rapid than in those birds whose wing movements are slower. The swallow had the most highly developed powers of flight of any of the forms used for experiment, and the disturbances were greatest in this bird. He draws the general conclusion that when one makes the same destructive lesion of the vestibule in each of a series of different forms of birds, the resulting disturbances of movement are greater the more difficult it is for the bird to maintain its equilibrium normally in one form of progression, and the greater or more rapid the degree of muscular coördination required for its successful execution.

Experimental work has been carried out in birds, and particularly in pigeons, in much greater detail. In general, there is torsion of the head to the injured side, sometimes leaning of the body to that side, inequalities of wing movements, the movements being less extensive and of less force on the injured side, and a loss of general muscular tonus on one side, when the lesion is unilateral. Rotation is attended by head movements, head nystagmus, as Breuer calls it, and ocular nystagmus, in normal birds. The reactions to rotation are lost after bilateral

vestibular lesions when the eyes are covered. There is also a great disturbance in the sense of position of the head, and Exhibit 1(g)(m) for a permanent loss of tonus of the neck muscles.

Exhibit 1(k) (1)(n) applied ice to the left posterior vertical canal of a pigeon and observed rapid movements of the eyeballs, the eyes being closed; when the pigeon attempted to move, there were rather feeble backward movements of the head and traces of ataxic gaiters. Continued cooling of both posterior vertical canals increased the backward movements of the head and the tendency to fall backward. Pendulum

TABLE I

Experiment	Action	Reaction	Condition	Reaction	After action	Explanation
Very marked	Positive					
Marked	Positive	Lower				
Moderate	Pigeon throws	Positive	Right. Positive			
Slight	Dies			Right. Pigeon dies		
Transitory	Dizzy			Quiet	Quiet	Excess. Has Positive Pigeon Quiet

movements of the head, occasionally passing over into circus movements of the body, also appeared. All effects disappeared in fifteen minutes after cooling was interrupted. The application of ice not to the left horizontal canal of a pigeon caused movement of the head to the right and closure of the eyelids. When the eyelids were opened there was strong deviation of the eyes to the right, with quick jerks to the left. Burning the right horizontal canal of the same bird caused movements of the head to the left, but less marked than above or had before turned to the right, and it soon returned to the mallow.

Considerable work has been done on mechanical stimulation of various separate canals in the pigeon. Exhibit 1(k), by means of the pneumatic hammer, set up pressure in a canal which was transmitted to the ampulla. There was, in the case of the horizontal canal, a movement of the head

to the opposite side in the plane of the canal. There is usually a return to the normal position immediately afterward. When the pressure is relieved, there is a movement of the head toward the side stimulated, but less marked than when the pressure was increased. In the case of the superior (vertical) canals, the movement following the relief of pressure is more marked than the movement following the increase of pressure. There is also movement of both eyes, the direction of the movement being the same in the pigeon as in the dogfish.

It should be pointed out that Maxwell's result on the transmission of pressure from the utricle to the ampullae of the various canals is not in any way inconsistent with Ewald's results. The facts seem to indicate that, wherever the pressure may arise, whether in the canals or in the utricle, its transmission to the ampullae is sufficient to excite the nerve endings within them. This is the important thing to be remembered in formulating any hypothesis of the normal mechanism of excitation of the ampullae.

Breuer (31) has repeated, on pigeons, Gaglio's (29) experiments on the application of cocaine to the vestibule. There is an agreement that simple anesthesia of the vestibule is attended by the same effects as its anatomical destruction. Breuer, however, disagrees with Gaglio in some matters of detail.

Borries (59), basing his conclusions partly upon experimental results in pigeons and partly upon clinical observations, thinks that the changes in the reaction to irrigation of the ears with water colder or warmer than the body,—the caloric nystagmus of the clinician,—with the changes in the position of the head are dependent upon the otoliths. I am unable to follow the reasoning involved in arriving at such a conclusion. While it has been shown by various experiments that we must probably attach more importance to the otoliths than we have heretofore, it has not yet been shown that the ampullae of the vestibule are without any functional importance. It seems much more likely that we must regard both parts of the vestibule as functioning together to bring about a common reaction.

Mammals. Although the first experimental observations on the effects of excitation of the vestibule were made by Darwin and Purkinje, perhaps even by Theophrastus, on the human subject, the greatest knowledge of the functions and manner of excitation of the vestibule has not been derived from observations upon the human or even upon mammals. As has been indicated, the nature of the problem changes somewhat when one reaches the birds and particularly the mammals.

Ferrigo, with which Parkyn deals, is a subjective thing and not wholly objective. Its manifestations goes far beyond the mere function of the vestibule and leads to a problem of reference peculiarly to the central nervous system itself. Parkyn's results fully bear out all essential observations that their contemporaries have to subscribe, but the problems of the central nervous system are such as to complicate matters quite here. Bolton (51) has given a peculiar instance of the phenomena of vertigo appearing under certain conditions in epilepsy.

The vestibular nystagmus mentioned before is a prominent feature of the effects of rotation in mammals. The detailed description of all forms of vestibular nystagmus is given by Ewald (52). The typical form of vestibular nystagmus, as observed in man and the higher mammals is not elicited by lesions of any other nervous mechanism, so far as my observations or knowledge goes, and it does not appear to extend form in lower vertebrates. Its manifestations however are such as could a priori be ascribed to disturbed vestibular function as a problem of the connections of the vestibular and vestibulo-spinal system within the central nervous system. As Ewald (52) states, when it is borne in mind that an injury to the vestibular nystagmus occurs, there is no other nystagmus following lesions of the cerebellum. The original statement that complete removal of the cerebellum in the dog does not prevent the appearance of vestibular nystagmus has been fully confirmed by Magnus for other mammals.

Bárány (53) showed that exactly the same phenomena of vertigo could be produced by electric stimulation of the vestibule, as result from rotation of an animal or man. The clinical application of the caloric method of stimulation of the vestibule has been mentioned by Babinski (55) and others.

All mammals, except man, show the tendency of the head to the injured side after unilateral vestibular lesions, with rolling movements to that side during the first few days. The tendency of the head is permanent, but the rolling movements and the nystagmus are transitory. The same effects are often seen accompanying abscesses of the mastoid cell in white mice and rats (56), guinea pigs and rabbits. I have never seen a case of an abscess of the labyrinth cell in dogs or cats. Walking or rotating in a circle to the injured side is also a transitory effect of a unilateral vestibular lesion.

After bilateral operations there is a slow swaying of the head from side to side, especially in rats, while dogs remain fast on the floor for many days afterward, refusing to walk or even stand. The decerebrate animal will stand even after removal of both vestibules (57). Walking

or running in a straight line soon becomes possible, and dogs or cats exhibit no noticeable defects of locomotion when on the ground. When the eyes are covered, the dog has difficulty in walking in a straight line, turning from side to side in an irregular way. Swimming with eyes open is a matter of great difficulty or even impossible in such an animal. It may swim on its side or back or turn downward toward the bottom of the pool. After unilateral operation a cat or dog will jump over the side of the cage or from a considerable height to the ground without falling or exhibiting any defects of movement. But a dog will not jump from even a moderate height to the floor without falling in a heap after bilateral operation. This observation was made by Schiff (68), and we have many times confirmed it. After one or two experiences, a dog will look over the edge of the table and whimper when food is held some distance from him, but he may refuse to jump to the floor in order to get it. Under conditions where the animal is deprived of the use of its proprioceptors and exteroceptors with the exception of sight, as in jumping or swimming, the effects of vestibular operations may be shown in dogs months afterward.

The deviation of the eyes is permanent in lower vertebrates following unilateral vestibular lesions, but it is merely transitory in higher forms. It has been found, also, that the nystagmus following rotation may be decreased or even abolished by repeated rotation of an animal or man on successive days (69), (70), (71). And with reduction of nystagmus there is a reduction in the degree of vertigo experienced in men. This does not seem to indicate any particular difference in the function of the vestibule of higher forms as compared with the lower, but to indicate that there is a considerable difference in the central nervous relations of the vestibular and oculomotor mechanism in higher forms. The problem again becomes one of the organization of the central system and its changes in phylogenetic development. And no place outside of the vestibule can one find any part of a proprioceptive mechanism which is circumscribed in location and which is capable of complete anatomical removal with so little damage to any contiguous nervous structures. For this reason as well as because of the very definite motor and postural disturbances following anatomical lesions the vestibular nerve endings offer a unique opportunity for the analysis of the central relationships of an extremely important proprioceptor. The problem is as yet unsolved.

Dodge (72) has made the only determinations of the threshold of stimulation of the vestibular endings known to me. The threshold

See Addison, F. and F. are proved for the nerve peripheries beginning at once. During normal activity the threshold seems to be higher as a change of 4° a second occurs normally before the subject notices any change in the rate of rotation.

Aside from the ordinary results of irritation, some other kinds of reaction have been noted in connection with the semicircular vestibule. Magnus and de Kleijn (73) showed that when a cat's head was lowered the vestibules being intact, the fore legs were flexed and the hind limbs extended; when the head was raised the fore limbs were extended and the hind limbs flexed. The numerous other papers of these authors are given in Griffith's (74) latest paper.

Wilson (74) has shown that right explosives may so affect the cochlear portion of the ear that hearing is irreversibly lost the vestibule may remain relatively unaffected. The history of the separation of the auditory and vestibular functions in the human ear is given by Gelle (75). Another noteworthy successful series of observations is that of Holmes (76) on the effect of gunshot injuries of the vestibulum. As Holmes points out, the effects of gunshot injuries in the human are more nearly comparable with the experimental results in animals than are the effects of ordinary pathological lesions.

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THE ACID-BASE EQUILIBRIUM IN SIMPLE TWO-PHASE SYSTEMS.

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The recognition of the association of the acid-base equilibrium with many, if not all, physiological processes has led many investigators of biological phenomena to study relationships between this equilibrium and particular systems in which they were interested. Such relationships are usually expressed in terms of the hydrogen ion concentration, the value of which enables one to calculate the ratio of the concentration of any free acid and its (ions) to the concentration of its salt. This applies, within ordinary limits, to a solution of a mixture of several acids and their salts with bases considered indistinguishably of the others. Many problems, for example the manner in which CO$_2$ exists in the blood, are, for all practical purposes, solved by this method.

The subject of this paper deals with a simple application of the method, together with the introduction of the distribution law, to two-phase systems of equilibrium. Many general and specific investigations of the acid-base equilibrium and its relation to the physicochemical behavior of proteins have given us a rational foundation for the description of these systems, and Loeb (1) has recently combined these foundations with the Donnan theory to interpret the colloidal properties of proteins. Considering the advance that has accompanied these researches, it is a curious fact that no similar general studies of the relationship between the acid-base equilibrium and lipoids have become widely known, if they have been attempted at all. That is to say, we are not aware of any treatment of the general equilibrium existing in the system, fat or fat solvent(s), fatty acid, salt, base, water.

This fact, and the desirability of an examination of this equilibrium, were mentioned to me by Professor L. J. Henderson, whom I particularly wish to thank for these suggestions, as well as for many opportunities he has afforded me in the past for studying the possibilities involved in the method of analysis to be used.

The general problem to be discussed in this paper deals with the distribution of a weak acid between an aqueous solution at various hydrogen ion concentrations and any other simple phase (in which the acid may exist) in equilibrium with this solution. Distribution, in this sense, refers to all the forms in which the acid may exist; *i.e.*, aggregated, dissociated, as salt, etc. For example, lactic acid distributes itself between amyl alcohol and water. On the other hand, Na lactate would be present almost entirely in the aqueous phase. Obviously, then, if NaOH is added to the system, lactic acid will migrate from the alcohol into the water following the formation of Na lactate in the aqueous phase. The problem is to relate the total distribution of acid with the hydrogen ion concentration, and to find out any other derived relations which may be of interest.

THEORETICAL.

It will be convenient to make clear certain crucial points involved in the two generalizations we shall use; namely, the mass law and the distribution law as they apply to the problem.

Henderson (2) derived the following equation from the mass law:

$$[\mathrm{H}] = \frac{[\mathrm{HA}]}{[\mathrm{BA}]} \times \frac{K}{\gamma}$$

and this equation has been repeatedly verified by experiment (see Clark's book (3)). Now in these experiments the apparent and obvious values for HA and BA, when substituted in the equation, lead to consistent results; and this fact leads to the conclusion that the addition of the salt of a weak acid to a solution containing the weak acid, does not effect any change in the concentration of the free undissociated acid. In other words, addition of BA results in a corresponding change in H, but no change in HA. Conversely, addition of HA results in a change

in H but not in BA. This statement is evidently true as a first approximation; in fact any deviation from this rule would certainly be very small compared to the magnitude of the changes the equation is designed to express, especially with weak acids, and in the range of usefulness of the equation. Evident as this may seem, it is emphasized because it leads to an apparent contradiction of a statement made by Nernst to which reference will be made later.

The distribution law (4) involves the proposition that the distribution ratio of any given molecular species between two phases is a constant independent of the presence of any other species except as these may modify the thermodynamic environment of the respective phases.

These two well known generalizations lead to the equations developed below. The calculations consist merely in taking a known amount of a weak acid, some of which exists as salt. The remainder, free undissociated acid, is distributed between two phases according to a known ratio. The concentration of free acid remaining in the aqueous phase divided by the concentration of its salt leads to a value for the hydrogen ion concentration. The salt formed is assumed to be present in the aqueous phase only. The solutions are supposed to be sufficiently dilute to come within the range of applicability of the mass law, and the volumes of the two phases are assumed to remain constant (unless otherwise specified), and the temperature is assumed to be constant. The calculations are so simple that we would hardly feel justified in presenting them were it not for the fact that the consequences involved in the equations developed are not directly obvious from the original assumptions and appear to be of some interest. Moreover, the general laws of physics and chemistry become increasingly important to the physiologist as they are applied more and more specifically to those special conditions which characterize the organism.

Three different cases to be designated A, B, and C will be treated, differing from each other in respect to the nature of the existence of the weak acid in the nonaqueous phase. The following notation will be used:

M = total amount of weak acid in gram molecules present in any two of solution in the system.

V_w = volume of aqueous phase, in liters.
V_s = volume of "second" phase, in liters.
HA_w = amount of free undissociated acid in aqueous phase, in gram molecules.
HA_s = total amount of weak acid in the second phase, in gram molecules.
BA = amount of salt of the weak acid, in gram molecules; which is equal to the amount of available base.
A' = amount of dissociated acid anions in aqueous phase, in gram molecules.
γ = degree of dissociation of the salt.

(Since by far the greater part of A' is derived from the dissociation of BA, Henderson showed that we can write $A' = BA\ \gamma$, without introducing any significant error. Furthermore, $M = HA_s + HA_w + BA$ very nearly.)

y = fraction of total acid existing in the aqueous phase $= \dfrac{HA_w + BA}{M}$

K = dissociation constant of the acid.

$K' = \dfrac{K}{\gamma}$

[H] = concentration of hydrogen ions, in gram molecules per liter of aqueous solution.

(Concentrations for other constituents will also be expressed by brackets.)

D = the distribution coefficient, applied here only to the molecular species $HA = \sqrt[n]{\dfrac{[HA_w]}{[HA_s]}}$

C = a new factor to be defined below, related to D.

$C' = C\dfrac{V_w}{M}$, a factor to be referred to as the distribution factor.

n = the number of molecules of HA in an aggregate existing in the second phase, commonly written in the form $(HA)_n$.

We can now turn to a simple case. In addition to the conditions enumerated in the preceding paragraph:

For Case A it is further specified that the second phase consists of an inert solvent (gas or liquid), and that the weak acid, HA, in this phase is neither associated nor dissociated to any significant extent. The conditions at equilibrium can be defined by the following equations.

From the mass law:

$$[H] \times \frac{A'}{V_w} = \frac{K\ HA_w}{V_w}; \text{ or } [H] \times A' = K\ HA_w. \qquad (1)$$

Since $A' = BA\ \gamma$, and $K = K'\ \gamma$, substituting we have:

$$[H] \times BA = K'\ HA_w \qquad \text{(Henderson's equation)}$$

For BA substituted as cation $pM = HA_m$. Definition of $\bar{y}$, and we have:

$$HA_m = \frac{\bar{y}\, M\,(II)}{K^{-n}\,(H)} \qquad (2)$$

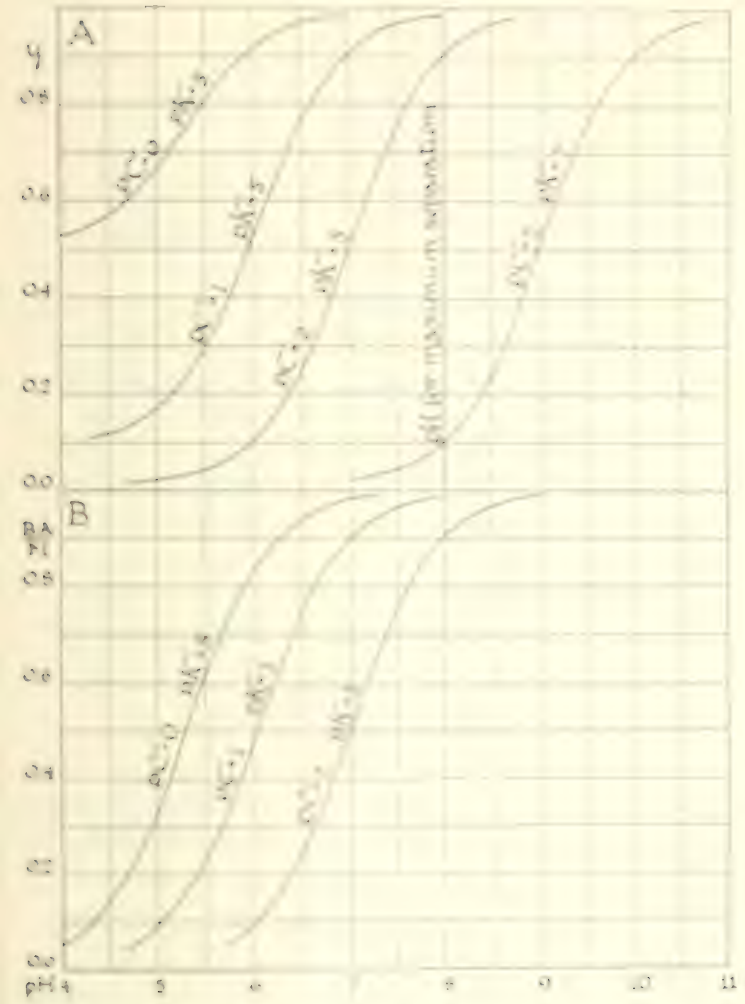

Fig. 1.

From the distribution law:

$\frac{[HA_w]}{[HA_s]}$ = a constant, D, when the nature of the phases is specified. We will define C by the ratio $\frac{HA_w}{HA_s}$. That is:

$$C = \frac{HA_w}{HA_s} = \frac{[HA_w]}{[HA_s]} \frac{V_w}{V_s} = \frac{V_w}{V_s} D \tag{3}$$

Case A.

In Case A, $n = 1$, and $C = C'$

From equation (3), $HA_w = C\ HA_s$, and from the definition of y, $HA_s = M\ (1 - y)$, therefore:

$$HA_w = C\ M\ (1 - y) \tag{4}$$

Combining equations (2) and (4),

$$\frac{y\ M\ [H]}{K' + [H]} = C\ M\ (1 - y), \text{ from which: } y = \frac{C\ (K' + [H])}{[H] + C\ (K' + [H])} \tag{5}$$

To find an expression for $\frac{BA}{M}$, subtitute the term $yM - BA$ for HA_w, in Henderson's equation, and we obtain:

$$\frac{BA}{M} = y \frac{K'}{K' + [H]} \tag{6}$$

and combining this equation with equation (5) to eliminate y, we get:

$$\frac{BA}{M} = \frac{CK'}{[H] + C\ (K' + [H])} \tag{7}$$

Case B.

Case B is similar to Case A, except that the weak acid is associated to the extent n in the second phase, and exists in the form $(HA)_n$ almost entirely. Then, as Nernst has shown:

$D = \frac{HA_w}{V_w} \times \frac{\sqrt[n]{V_s}}{\sqrt[n]{HA_s}}$ = a constant. Since the volumes are constants,

$\frac{HA_w}{\sqrt[n]{HA_s}}$ = a constant = C, which applies only for the given volumes.

(If D is known from previous experiments, C, for any given volumes can be found by multiplying D by $\frac{V_w}{\sqrt[n]{V_s}}$).

It follows that (compare equation (4.1))

$$\Pi A_0 = C \sqrt[n]{\Pi A_0} = C \sqrt[n]{R(1-\gamma)} \qquad (5)$$

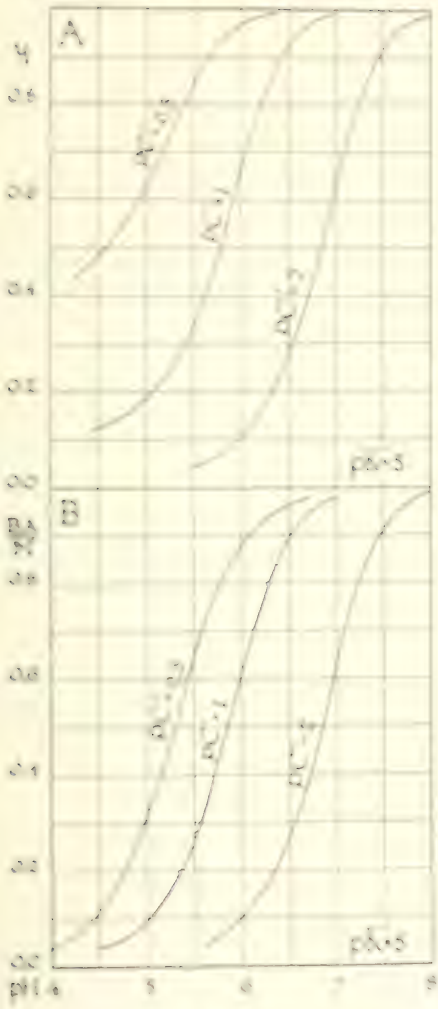

FIG. 2.

Combining equation (8) with equation (2) to eliminate the term HA_w we obtain:

$$\frac{y}{\sqrt[n]{1-y}} = \frac{\sqrt[n]{M\,C\,(K'+[H])}}{M\,[H]}$$; C' may be substituted for the term $C\frac{\sqrt[n]{M}}{M}$

This equation can also be written: $$[H] = \frac{C'\,K'\,\sqrt[n]{1-y}}{y - C'\sqrt[n]{1-y}} \qquad (9)$$

Also: $$\frac{BA}{M} = y\,\frac{K'}{K' + [H]} \qquad \text{(compare with equation (6))} \qquad (10)$$

Case C.

For Case C it is specified that the second phase shall consist of pure acid only (*i.e.*, as pure solid or liquid). An acid gas at constant partial pressure also satisfies the requirements.

In this case the acid can be considered as infinitely associated, $n = \infty$.

$$\text{"}D\text{"} = \frac{HA_a}{V_a} \times \frac{\sqrt[\infty]{V_s}}{\sqrt[\infty]{HA_s}} = \frac{HA_w}{V_w} = \text{the solubility of the acid in unit volume.}$$

$$C = \frac{HA_w}{\sqrt[\infty]{HA_s}} = HA_w, \text{ so long as both phases are present.}$$

$$C' = C\,\frac{\sqrt[\infty]{M}}{M} = \frac{HA_w}{M}$$

Substituting $C'\,M$ for HA_w in Henderson's equation, we obtain:

$$\frac{BA}{M} = \frac{C'\,K'}{[H]} \qquad (11)$$

Evidently, $yM = C + BA$

and, $$y = C' + \frac{C'\,K'}{[H]} = \frac{C'\,(K' + [H])}{[H]} \qquad (12)$$

All the relations developed are for equilibrium conditions.

The peculiar characteristics of these cases are defined by an additional constant, designated C'. Since this constant will be referred to below, it will be convenient to mention, in words, the method of determining its value.

For any given system, the volume of the phases being kept constant, C measures the ratio of the *amount* of free weak acid in the aqueous phase to the n^{th} root of the total *amount* in the second phase. The value is, of

[illegible] determined [illegible] under conditions which [illegible] present. For a weak acid, the molecule in the aqueous phase exists almost entirely in undissociated form, HA_w, when no buffer is present. In the second phase the acid is assumed to be almost entirely in the form HA_o. Hence the justification for writing the ratio determined for a neutral acid as $\frac{HA_w}{\sqrt{HA_o}}$, remembering that HA_w refers to the particular molecular species HA_w whereas HA_o refers to the total acid in the second phase.

Theoretical Discussion

Before discussing the special characteristics of these systems, we should consider a prediction advanced by Nernst (4) and quoted by Lewis (5). Nernst in his paper concluded that addition of HCl or of Na salicylate to a dilute aqueous solution of salicylic acid in equilibrium with a portion of benzene would *largely* increase the concentration of salicylic acid in the benzene phase (and, of course, in the aqueous phase). In the equations that have just been given, however, no account is taken of any such effect, since it is very small for weak acids and can be neglected when compared to the effects which have been or will be described. Salicylic acid in normal concentration is about 3 per cent dissociated; increasing the hydrogen ion concentration to any possible extent would never result in more than about a 3 per cent increase in the concentration of the undissociated portion. The same reasoning applies to the effect of adding Na salicylate. The maximum "Nernst effect" is 1 per cent (or less) if the ratio of the concentration of free weak acid to the value of its dissociation constant, *i.e.*, $\frac{(HA)}{K}$, is 10^2 (or greater).

Accordingly, it can be said that the equations given represent the actual conditions underlying the important changes in the distribution of weak acids. It should be added that no account has been taken of changes which take place in respect to the nature of the phases. For instance, an aqueous solution saturated with $(NH_4)_2SO_4$ cannot be substituted for a dilute aqueous solution if the constants, determined for the aqueous solution, are expected to hold. Similarly, salt formation in the aqueous phase, presumably affects the nature of this phase, and the distribution factors for acids may change considerably under the conditions which our equations are supposed to describe. In

any given case it should also be remembered that the salt formed may be soluble in the second phase.

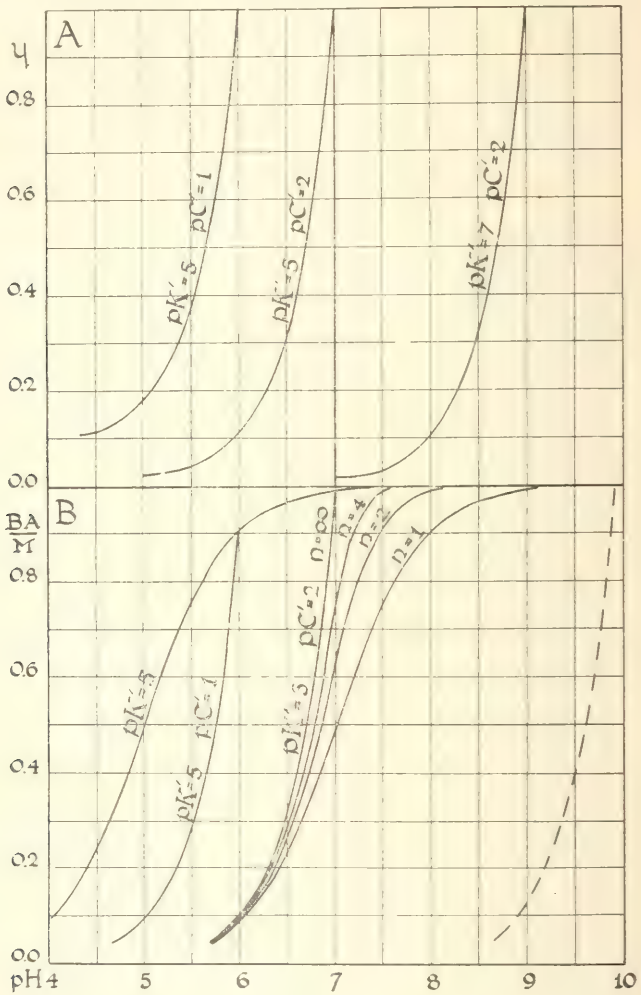

Fig. 3.

We can now examine any characteristics of the systems which appear to be of interest from the point of view of the acid-base equilibrium.

1. The effect of the second phase on the maximum molecular buffer value of a weak acid (Van Slyke's β_{max}).

In Fig. 3 *B* a comparison can be made between the slopes of the four curves at the right of the figure. These curves represent acids whose dissociation constants are represented by the value $pK' = 5$ (pX is short for $\log \frac{1}{X}$, and whose distribution factors are represented by $pC' = 2$. The curve marked $n = \infty$ illustrates the titration of a solution saturated with respect to the acid and containing a large part of undissolved acid besides. This system gives the highest buffer value. The curve $n = 1$ gives the same slope as would be obtained for a curve representing the titration of any weak monovalent acid in simple aqueous solution, for example for the curve on the left of the figure. When $n = 2$, or $n = 1$, that is, for Case B, the slope is intermediate between the slopes for Cases A and C. (The slope for Case B is difficult to define since at any given point and with a constant value for n it depends in a somewhat complicated manner on the value of C' if C' is greater than about 0.2.)

2. The effect of the second phase on the pH at which the buffer value is a maximum. (It is suggested that this point be designated pH(β_{max}).)

It will be remembered, as was first shown by Henderson, that in simple aqueous solution a weak acid exhibits its maximum buffer action at a pH equal to pK'. This pH occurs at a stage in a titration when half of the weak acid has been "neutralized" by base, i.e., when $HA = BA$.

When distribution takes place between two phases, however, pH(βmax) is displaced by a certain definite amount. When C' is small for all cases, pH(β_{max}) = $p(C'K')$. This value pH(β_{max}), that measures the pH at which the change in the distribution of an acid is a maximum, or $\frac{dp}{dpH}$ is a maximum when pH = $p(C'K')$. If C' is not small, then we have the following relations:

For Case A: pH(β_{max}) = $p\left(\frac{C'K'}{1 + C'}\right)$, and here $\frac{HA}{M} = 0.5$

For Case C: $\mathrm{pH}(\beta max) = p\left(\frac{C'K'}{1-C'}\right)$, and here $\frac{BA}{M} = \frac{C'}{1-C'}$

For Case B: $\mathrm{pH}(\beta max)$ has a value between the two values given above.

When C' is small, at the point $\mathrm{pH}(Bmax)$, $\frac{BA}{M} = 0.62$

when $n = 2$, and 0.72 when $n = 4$.

3. The effect of the second phase on the apparent strength of a weak acid.

No general rule can be laid down for the three cases. For any case it is evident that, if distribution takes place, the concentration of the acid is effectively diminished, as far as the aqueous phase is concerned. For Case A we can make the more precise statement: Those properties of a weak acid in simple aqueous solution which are characterized by the dissociation constant K, are characterized by $\frac{C'K}{1+C'}$ (or $C'K$ if C' is small) when the same acid is subject to distribution effects described under Case A and defined by the constant C'. This statement involves the relations brought out in the two preceding sections.

If C' is small, all the effects described can be expressed quantitatively, for all the cases discussed, and for the given point $\mathrm{pH}(\beta max)$, by substituting the value $pC'K'$ (when distribution takes place) for the value pK' (applying to simple aqueous solution). In other words, if an acid is distributed between two phases, it behaves as a much weaker acid, its buffer value is sometimes changed, and the pH at which the buffer value is a maximum is shifted toward the alkaline side. (These statements, and certain relations developed, do not apply to conditions where C' is greater than 1.)

EXPERIMENTAL.

To test the validity of the foregoing equations simple systems, typifying the three cases discussed, were selected. It happens that other systems which would exhibit more strikingly the peculiar characteristics of the two-phase equilibrium are more apt to involve, perhaps by their very nature, complicating factors and analytical or other experimental difficulties.

As an example of Case A, the distribution of lactic acid between amyl alcohol and aqueous solutions was studied. Benzoic acid distributed between benzene and aqueous solutions afforded an example of Case B, and crystalline benzoic acid in equilibrium with an aqueous solution corresponds to Case C.

Case A.

The distribution ratio of lactic acid (Merck's) between equal volumes of amyl alcohol and water was determined at three concentrations (1, 0.2, and 0.02 N normality considerations with respect to the volume of one phase) and found to be a simple constant, $\frac{HA_o}{HA_w} = 1.9$. In further experiments the volume of amyl alcohol was four times greater than the volume of water, hence the value for the distribution factor, $1' = 0' = 0.475$.

TABLE I

$\frac{y-x}{x}$	x	$\frac{HA}{M}$	pH
0.974	0.506	0.0	
0.243	0.403	0.5	3.39
0.233	0.402	0.5	3.97
0.193	0.393	0.75	4.46
0.108	0.452	0.5	6.78

80 cc. portions of amyl alcohol, containing lactic acid (0.05 N volume concentration), were shaken up in separate cylinders with 20 cc. portions of aqueous solutions of NaOH of varying concentrations. After equilibrium had been attained, the acid remaining in the alcohol layer was estimated by titration of a 10 cc. portion diluted with ethyl alcohol with aqueous NaOH, using phenolphthalein as an indicator. The pH of the aqueous layer was then determined electrometrically. The results are given in Table I. x is the fraction of the total acid in the alcohol layer at equilibrium, y is the total lactic acid or lactate in the aqueous portion. $\frac{HA}{M}$ is the amount of NaOH originally present in the aqueous portion expressed as the fraction of the amount required to neutralize all the acid present.

To compare these results with calculated values it is necessary to know the value of pK' for lactic acid. This was determined, for our sample of the acid, by measuring the pH of a 0.2 N solution of the acid to which had been added half its volume of a 0.2 N solution of NaOH. The observed value was pH = 3.54 = pK'.

Using the values for C' and pK' which have been given, the curves in Figs. 4 *A* and 4 *B* have been calculated. The distribution curve, Fig. 4 *A*, is calculated from equation (5). In Fig. 4 *B*, Curve 1 is the theoretical titration curve for lactic acid in aqueous solution, and Curve 2 is the curve which applies to the experiment, calculated by means of equation (7). The x's, plotted from the observed data for the distribution curve, are taken from values for y and pH in the table, and for the titration curve they are taken from the values for $\frac{BA}{M}$ and pH.

Case B.

It is well known that the distribution relations of benzoic acid between benzene and water can be calculated on the basis that benzoic acid in benzene exists in the form $(HA)_2$. Hence $\frac{HA_w}{\sqrt{HA_s}}$ is a constant ratio = C (which applies only to given volumes of the two phases). To determine the value of C, 20 cc. of benzene, containing 0.002 gm. molecules of benzoic acid, were shaken up with 20 cc. of water. At equilibrium 0.000152 gm.-molecules of the acid were in the water phase, and 0.00185 in the benzene. Hence

$$C' = C\frac{\sqrt{M}}{M} = \frac{0.000152}{\sqrt{0.00185}} \times \frac{\sqrt{0.0002}}{0.0002} = 0.079$$

The same volumes of benzene and water, and the same amount of benzoic acid were used in the series of experiments which are summarized in Table II. In the experiments marked with an asterisk (*) NaOH was present in the aqueous portion; in those marked with a dagger (†) buffer salts were also present. The latter do not yield data for the titration curve, but show that the distribution curve can be expressed as a function of the pH and is independent of the nature of the salts which may in part determine the pH.

pK' for benzoic acid was determined as for lactic acid except that the concentration had to be sufficiently low (0.02 *N*) to ensure complete solution of the acid. The value for pK' was found to be 4.05.

Figs. 4 *C* and 4 *D* show the theoretical curves and the observed points. Curve 1, Fig. 4 *D*, is the theoretical curve for the titration of benzoic acid in aqueous solution. Curve 3 the theoretical curve for the mean of experiments of Table II. These curves are calculated from equations (9) and (10).

TABLE II.

$\frac{n}{N}$ = ϕ	x	$\frac{\Delta x}{\Delta \phi}$	pH
0.012*	0.0022	0.0	3.40
0.860*	0.159	0.0	4.04
0.750*	0.333	0.15	4.37
0.610†	0.564		4.78
0.575*	0.625	0.43	4.91
0.334†	0.800		5.30
0.255*	0.846	0.25	5.55
0.150†	0.926		6.30
0.085*	0.955	0.05	6.77

TABLE III.

Total acid M = 0.005, solubility = 0.0025.

	$\frac{n}{M}$	pH
Crystals present	0.1	3.18
" "	0.2	3.64
" "	0.5	4.45
All dissolved	2.5	4.85

Case C.

Portions of benzoic acid (0.005 gm. molecules each) were introduced into 50 cc. volumetric flasks which had been filled up to the mark with aqueous solutions containing various amounts of NaOH. After equilibrium had been attained (*i.e.*, after gentle warming and cooling to 25°) the pH of samples from each flask was determined colorimetrically (checked by several individuals) using Clark's series of standards, freshly made up according to

the procedure given in Clark's book. The solubility (C) of benzoic acid given in the tables is 0.00119 gm. molecules in 50 cc.

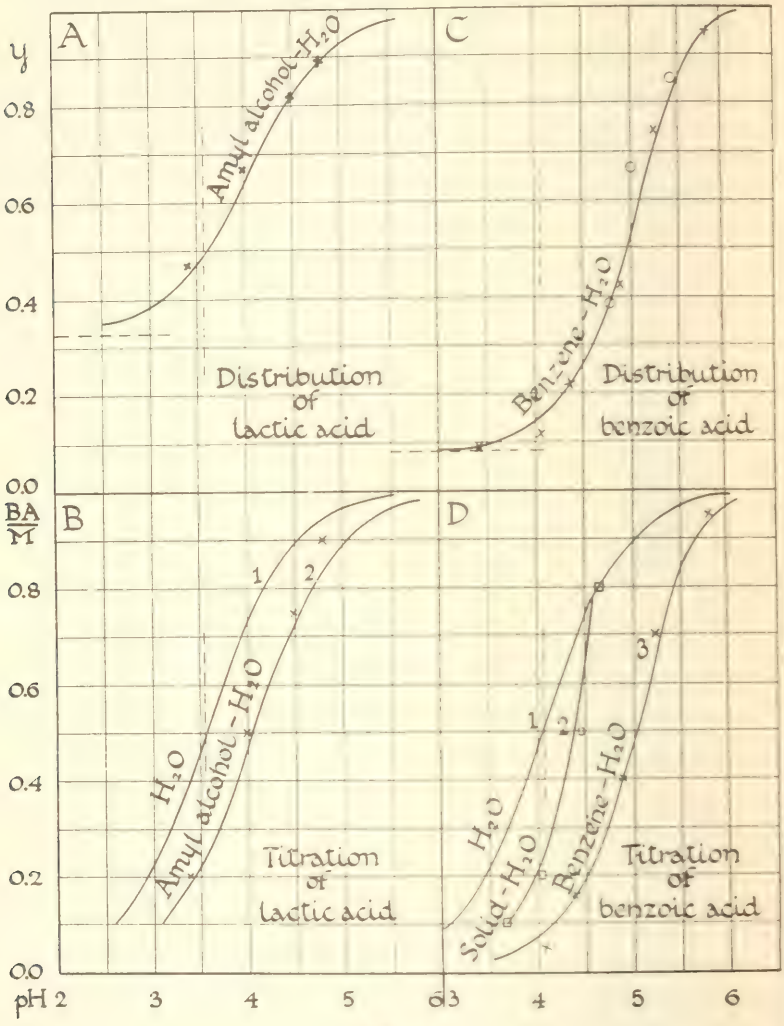

Fig. 4.

at 20°, hence $C' = \frac{0.00119}{0.00500} = 0.238$, and, as before, $pK' = 4.05$.

Table III gives the results observed.

Curve 2, Fig. 4 *B*, is the calculated curve, from equation (11), representing the relations which obtain when both phases are present. At the point $pH = p\left(\frac{CK}{1+C}\right)$, that is pH = 4.55 in this example, the neutral point disappears and the relations which hold for the titration in simple aqueous solution (one phase) are sufficient. The observed points are marked by squares in the figure. It is interesting to note that the point $pH = p\left(\frac{CK}{1+C}\right)$ also represents the pH at which the maximum buffer value of the acid is exhibited (although, actually at this point, the high buffer value is only effective in one direction). This is also, obviously, the pH at which the acid begins to precipitate when a strong acid is added to a solution of the salt.

It appears, from these experiments, that no fundamental error is involved in the equations which have been submitted, nor would this have been expected, since the equations are based on simple generalizations which are easily combined for the purpose in hand. No claim is made that these equations are new in their most useful form, but they serve, at any rate, to indicate the general efforts to be encountered when dealing with simple two-phase systems.

Further Considerations.

It remains for us to suggest the significance, if any, of the foregoing results, and to point out a few applications which may be of use.

A glance at the curves makes it apparent that we can separate two acids if their dissociation constants are sufficiently different, even though their distribution coefficients and solubilities in various solvents are identical. The separation becomes more and more complete as the value $pC'K'$ for the acids differ more and more from each other. The value of C' should be small, but this can usually be provided for by increasing the volume of the second phase. Fig. 5 *A*, considering the two curves at the right, will illustrate the method of separation, which is based on the fact that the pH of the aqueous phase can be controlled by means of a suitable indicator and that a large shift of acid from one phase into another occurs within a relatively narrow range of

variation of pH. The two acids, the behaviors of which are represented by the curves, have values of $pC'K'$ equal to 7 and 9. The pH at which maximum separation is obtained is therefore 8. Suppose that the acids are dissolved in water, and that they are to be separated by means of benzene as the second solvent.

Equilibrium is first attained between the two phases, and alkali added until the pH of the aqueous phase reaches 8. The two phases are shaken up together again, acid passes into the aqueous portion, and more alkali is needed to bring the pH back to 8. When equilibrium is obtained at a pH of 8, the two phases are separated. At this stage we should have 90 per cent of the strong acid in the aqueous phase and 10 per cent of the weaker acid. If the aqueous phase is now extracted with a fresh portion of benzene, and the pH at equilibrium brought to 8 (this time by adding HCl), then 81 per cent of the strong acid will remain in the aqueous phase and 1 per cent of the weak acid. One more extraction will yield 72 per cent of the original amount of the strong acid and 0.1 per cent of the weak acid. The weak acid may be similarly purified by extracting the first separated portion of benzene with fresh portions of water, always bringing the system to equilibrium at a pH of 8 to obtain the most efficient separation.

The same method can be used to separate two acids by precipitation from solutions of their salts. This is illustrated by the two curves at the right of Fig. 3 *A*. As strong acid, HCl, is added to such a solution, the weak acid whose $pC'K'$ value is 9 will begin to precipitate when the pH of the solution drops to 9, and will continue to precipitate pure until the pH has dropped to 7. At this stage the stronger acid would begin to precipitate, and the time for separation is reached. 98 per cent of the weaker acid can be filtered off, presumably quite pure; but further efforts to purify the stronger acid will be useless, because the curve for the weaker acid which remains now follows the curve for the stronger acid. What is gained in favor of the purification of the weaker acid is lost in respect to the stronger acid, as far as this precipitation method is concerned.

Similar principles would apply to the fractional precipitation of insoluble metallic salts of weak organic acids, and it seems that a judicious use of indicators in such procedures might serve to make certain separations more precise.

Since the distinctive feature of the evidence we have been discussing are most evident when small values of C are considered we would expect that the principle discussed would be preferably tested by attempts to the study of systems involving the higher fatty acids. As these acids are extremely insoluble in water, we would expect to obtain a titration curve such as the dotted curve in Fig. 3 *B*, if we assume that the soap formed is soluble in water. Under these conditions the insoluble fatty acid would presumably be emulsified in the soap solution and would give to these solutions their colloidal properties. The possibility that the solubility of the fatty acid would be significantly increased in this condition should not be overlooked. The value for pK'_a (or which the inclusion curve is drawn is taken from McBain's data (7) for the pH of a "solution" 1 *N* in respect to Na palmitate and palmitic acid mols. (This gives a value of 8.9 for pK'_a.)

Studying the distribution of oleic acid and oleate between benzene and water at various equilibrium hydrogen ion concentrations, I have found that when the total oleic acid is equally distributed between the two phases the pH of the aqueous phase is 9.0. If the pH is less than 9.0 the oleate in the aqueous phase is practically zero, i.e. there is such a complete precipitation of the oleate by the acid as to lower the surface tension of the water phase. Hardy and Peters (8) have studied the surface tension at the interface of an aqueous solution at various pH's and carbon tetrachloride. They found the surface tension at the interface to be a linear function of the pH over a considerable range, dropping to zero as the pH reached the value 9.

McBain alludes to the fact that CO_2 will decompose soap solutions, so that obviously nearly all the fatty acid may be removed. He adds that the equilibrium obtained have not been subject to quantitative study. Since the pK' values for higher fatty acids are thought to be of the order 5, the action of CO_2 might appear at first sight to be somewhat anomalous; but from what has been said it will be noticed that the effective strength of these acids is measured by the value $pC'K'$ (roughly, a value of the order of 10 for 0.1 *N* solutions). In short the higher fatty acids, in aqueous solution at least, behave like acids very much weaker than H.HCl's.

The possibility suggests itself that the solubility of the soaps is limited. If this is true, then, as long as undissolved soap and undissolved acid are present in equilibrium with a given solution, we have $\frac{HA}{BA}$ = a constant. Addition of a portion of HCl to the system results in a transfer of fatty acid from the undissolved soap to the undissolved fatty acid and leaves the solution unchanged (at equilibrium) except for the formation of a certain amount of NaCl. Hence there is a unique hydrogen ion concentration at which the three phases can exist in equilibrium, and we have a system which might truly be termed a perfect buffer system.

Another application of the relations involved in the distribution of acids suggests itself from an analogy which can be drawn between the systems we have reviewed and typical systems which have been used to investigate the Donnan equilibrium. One essential feature of this equilibrium is that it is a two-phase equilibrium, and another is that one reacting ion is present in one phase only (more precisely, one ionic species cannot pass from one phase to the other). These two features are closely paralleled by a system consisting of two aqueous solutions separated

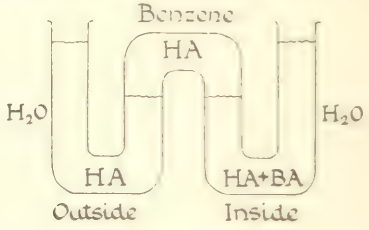

Fig. 5.

by a layer of some immiscible solvent such as benzene, the one solution containing an acid (soluble in benzene) and the other containing the same acid in equivalent concentration plus some of the salt of the acid. The benzene layer will contain only the free acid, and the system will be in equilibrium with respect to the acid. A neutral substance, such as sugar, can be added to the solution which contains only the free acid, and the system will then be in osmotic equilibrium.

In such a system the Na^+ ion corresponds to the indiffusible ion, and, to compare this system with those that have been investigated, we can call the solution containing the salt of the acid the inside solution (Lloyd had a solution containing protein gelatin ions) and we can call the other the outside solution.

At equilibrium the following relations will hold:

$$\underset{\text{Outside}}{H} \times \underset{\text{Outside}}{A^-} = \underset{\text{Membrane}}{K} \times \underset{\text{Membrane}}{HA_m} = \underset{\text{Membrane}}{[H]_m} \times \underset{\text{Membrane}}{A^-_m} = \underset{\text{Inside}}{H} \times \underset{\text{Inside}}{A^-}$$

which might be represented diagrammatically by the figure

$$H \times A = HA \rightleftharpoons K \rightleftharpoons HA \rightleftharpoons H \times A$$

Evidently the inside solution possesses the greatest osmotic pressure, a smaller concentration of acid anions, and a lower hydrogen ion concentration. Further work will be attempted with this system as it is thought that the system described offers a relatively simple case of the Donnan equilibrium, and offers an opportunity for measuring such properties as the interfacial surface tensions, and the potential charges of isolated droplets in either of the two aqueous solutions with which these droplets are in equilibrium.

In physiology it seems that a use could be found for the equations that have been developed here. The conditions governing the physicochemical behavior of fats, fatty acids, and soaps in the intestine are in need of more precise definition. Very roughly we might hazard the prediction that soaps from higher fatty acids begins to be formed in the intestine when the pH first reached the value 9. For a more exact application we must accumulate more data, for instance data to define the conditions under which calcium soaps, slightly soluble in fat, are formed.

This brings up the problem of the Na:Ca ratio in the blood. Are calcium soaps present dissolved in blood fat, and is the distribution of calcium between blood fat and plasma (and coincidently the Ca ion concentration in the plasma) affected by physiological changes of pH, should such a distribution really exist? Calcium salts in general, so many of which are insoluble, offer interesting material for physiological investigation from the point of view of the acid-base equilibrium, especially since the

physical state of calcium compounds must have a bearing on calcium assimilation from the intestine. In this connection reference should be made to an article by Wendt and Clarke (9) which has just been published, and which gives the titration curve for the neutralization of H_3PO_4 by $Ca(OH)_2$. At first sight the result is somewhat unexpected, and can be summed up approximately by saying that as $Ca(OH)_2$ is added to the solution of H_3PO_4 there is a sudden change of pH when one-third of an equivalent of base has been added. Soon after, a precipitate of tricalcium phosphate (perhaps of dicalcium phosphate also) appears and the pH remains practically unchanged at a value about 5.8 until 0.8 equivalents of base have been added, (no sudden change occurring when two-thirds of an equivalent are present). At this point the solution becomes gradually more alkaline, and finally suddenly much more alkaline at the end-point. Saturated lime water and 0.05 N H_3PO_4 were the solutions used for the titration. Equilibrium in this system, involving three phases or at least two, was only reached after considerable time. Presumably the value 5.8, for the pH at which the buffer action was a maximum, would shift toward the alkaline side if lower concentrations were used. While the pH remained constant, it is possible that addition of $Ca(OH)_2$ was causing little or no change in the *solution*, but merely a transfer of precipitated dicalcium phosphate to precipitated tricalcium phosphate. The solution, saturated with respect to the two salts, is a perfect buffer with a pH 5.8 as long as the three phases are present.

SUMMARY.

The distribution law and the law of mass action (Henderson's equation) have been combined to yield equations expressing the relation between the distribution of a weak acid between two phases, one of which is an aqueous solution, and the pH of the aqueous phase.

By the use of an easily determined constant, designated C', we can in general express by the value $C'K$ certain properties of a weak acid subject to distribution between two phases; namely, those properties which are defined by the value of K, the dissociation constant, when the acid exists in simple aqueous solution.

Some consequences deducible from the equations are discussed in some detail in respect to their bearing on the acid-base equilibrium in general. Thus the buffer value and the change in the actual distribution of total acid between the two phases are a maximum when the pH of the aqueous phase is equal to $pK'K''$ (when K'' is small). The value $pK'K''$ measures the apparent strength of the acid under the conditions specified, and it defines the pH at which a solid(s) insoluble weak acid begins to precipitate when a strong acid is added to a solution of the salt of the weak acid.

Experimental data are given to support the main conclusions.

Suggestions are made concerning the application of the equations to problems involving the separation of weak organic acids from one another. Speculations are advanced concerning possible relationships between some substances and systems described in this paper. A relation between the Henriques equilibrium and the equilibria discussed in the paper (for that equilibria involved are closely related) is described.

Some problems in physiology which might reasonably be approached from the point of view expressed here are suggested.

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A STUDY OF THE RELATION OF AFFERENT IMPULSES TO THE ACTIVITY OF THE CENTRAL CARDIO-VASCULAR NERVOUS MECHANISM

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Experiments have shown that there is a close relationship between impulses arising from experimental stimulation over specific afferent nerves and changes in heart rate and blood pressure. The question arises whether the maintenance of a certain degree of activity of the heart and blood vessels as manifested by the maintenance of blood pressure, is dependent upon afferent impulses in any degree or wholly independent of them. In other words, is the maintenance of blood pressure a reflex or an automatic function.

There is no method generally employed for eliminating the afferent pathway from the heart to the central nervous system while leaving the efferent path intact. It is possible, however, to leave the afferent path intact and eliminate temporarily a part of the efferent pathway through the action of such drugs as atropine.

One may divide one or both of the vagi anatomically and note the changes in heart rate, or one may eliminate the efferent fibers of the vagi by the administration of atropine, leaving the afferent fibers intact, but neither method really gives any clear answer to the question whether the action of the vagus under normal conditions is reflex or automatic. The problem is further complicated by the presence of the accelerator fibers, and the effect of the elimination of the vagi may conceivably differ according to whether the accelerators have or have not previously been thrown out. There are, then, several possible conditions under which the effect of elimination of the cardiac nerves may be studied.

ANATOMICAL. 1. Division of both vagi, with the accelerators intact. This would include depressor fibers in some animals, but not in the rabbit.

2. Division of both vagi after previous removal of the stellate ganglia.

3. Atropine, vagi intact and accelerators intact.

5. Heart: vagi intact but with nervous control of the skeletal muscles.

6. Section of accelerans, vagi intact.

7. Section of accelerans after division of both vagi.

8. Section of accelerans after administration of atropine.

The observations of all these conditions become necessary because of the possibility that the action of the anesthetics may be related to afferent impulses over the vagus since the existence of other than afferent cardiac nerves is doubtful. If efferent cardiac fibers do run in the accelerans, many conditions are introduced, all of which demand analysis. And to the various procedures given above, we must add division of the dorsal roots of the spinal nerves.

It may be remarked in passing that no complete experimental analysis of the problem has yet been made. And by the same token no clear answer can as yet be given to the question of the functional relation of afferent to efferent roots of the cardiovascular system.

Just as it was necessary to inquire into the relations of afferent to efferent roots in the matter of the nervous control of the skeletal muscles in order to decide which of their responses were reflex and which automatic in nature, so we must go over the relation of afferent roots to efferent roots in the study of the cardiovascular reactions. For purposes of simplicity and convenience in handling, we may consider separately the cardiac and vascular reactions, and then later try to get at the reciprocal functions of the two parts of the nerve mechanism.

It has been shown that stimulation of the central end of the vagus, when one is divided and the other intact, slows the heart as a rule, although there is sometimes acceleration of the heart instead. It has been shown also that the vagus is the only afferent nerve (stimulation of which will consistently slow the heart. There are both afferent and efferent fibers in the vagus and it has been impossible up to the present to separate these anatomically. The determination of the relation of one set of fibers to the activity of the other must be arrived at by some other more round-about and possibly less certain method.

The usual effect of section of both vagi is an increase in the rate of the heart and a rise of blood pressure. This simple experiment does not, however, throw much light on the question of whether the normal action of the vagus in keeping the heart rate down is reflex or automatic in character. In order to determine this point, further experimentation is necessary. It becomes necessary, therefore, to observe the conduct of the heart when the vagus is severed under a number of different conditions, which [illegible]

Statement of problem. Sherrington (1) has considered a funda mental characteristic of the responses of the central nervous system under the caption of the relation of afferent to efferent roots. The greater volume of work on this relationship has been devoted to the conditions prevailing in the somatic rather than in the visceral sensory and motor systems. While the responses of the somatic system are necessary for locomotion and other activities, and certain reactions of the organism to some conditions or changes of conditions in the external environment, the responses of the visceral system are necessary for the maintenance of the constancy of the internal conditions of the higher organism, such as systemic blood pressure, as well as for the adjustment of internal conditions in relation to the changes of the external conditions. The question arises whether the responses of the visceral system involve this relationship of afferent to efferent roots to the same degree as the somatic systems.

Sherrington considers this question to some extent in his chapter (loc. cit.) but the degree to which this relationship holds in such a nervous mechanism as that for the control of respiration or blood pressure is not answered. Nor does one find this answer in any of the papers so far published. It is evident that for the proper understanding of the function and manner of action of any of these visceral systems, the ascertainment of the degree to which the relation of afferent to efferent roots holds is a necessary preliminary.

It may be pointed out here that just as the changes of internal conditions, practically independent of afferent impulses, may affect the respiratory rate, so it is possible that changes of internal conditions which are not primarily related to afferent nervous impulses may affect cardio-vascular activity. The relation of afferent to efferent roots may then be masked or modified by other internal conditions in the case of both of these systems, and the answer to our question may at first involve the indirect quantitative estimation of a number of factors in an equation rather than the direct determination of one of them.

Historical. Beginning with the discovery by the Weber brothers of inhibition of the heart by applying one electrode to the spinal cord at the level of the 4th or 6th vertebra and the other to the nostril of a frog, and the later discovery of an inhibitory center by the same workers and others, the question as to whether this center and the vagi could be affected reflexly or not has been very much under discussion. Even after the vagus center had been definitely and minutely located in the medulla, very little progress had been made in regard to this question.

As a result, the mechanism of the nervous control of the entire cardio-vascular system was more or less in the dark.

That the vagus center may be reflexly stimulated by stimulation of various afferent nerves, and that vagal tone may be altered by the blood itself has been shown. That psychical and emotional states could change vagal tone was also shown by many workers. By stimulation of the cerebrum itself marked changes in the pulse rate may occur. But all this work did not show whether the tone maintained by the vagus center is kept up reflexly by constant instreamings of afferent impulses, or whether the center had the power of automatism. These experiments do not, for instance, indicate what effects changes in the internal environment of the organism may produce on the center.

That changes in the internal environment of an organism may have profound effects on pulse rate and blood pressure of an animal is very clearly shown by the changes in heart rate which follow deep inspiration and deep expiration. At the end of deep inspiration a marked increase in heart rate is observed as well as a marked increase in arterial tension; while a marked decrease in heart rate and a fall of arterial tension is observed at the close of deep expiration. The effect of any increased muscular work will change the internal environment to a certain degree, and this change may be accompanied by very great changes in the cardio-vascular system. This phase of the question has been largely overlooked by previous workers as well as by those who, until lately, have been working on the nervous mechanism of respiration. That vagal tone is maintained reflexly has been shown by some workers, such as Soltman, but the evidence presented by them is neither very clear nor very conclusive. In a similar manner evidence has been presented against this view which also lacks in conclusiveness. There is the work of Hill who makes the supposition that the terminations of the afferent nerves on the inner wall of the heart may be stimulated by intracardiac pressure and thus control the centers of circulation. That this view is perfectly tenable can be shown by the experiments of Johanssen (2) and many others who, after section of all the cardiac nerves, observed a rise of arterial tension and an increased heart rate on compression of the abdominal aorta. In the absence of the afferent pathways no stimulation arising from pressure in the heart can be effective reflexly. If however all the cardiac nerves are intact, Marey (3) noted that the compression of the abdominal aorta is followed by a decreased heart rate. Whether the central mechanism of the vagi is in this case affected by afferent impulses or by the increased intracranial pressure cannot be

determined. The heart, through the depressor nerves, has afferent connections with the central mechanism and by this means it is perfectly possible for it to control reflexly its own rate.

Sherrington (4) has sectioned all the dorsal roots of an animal in which he was recording blood pressure without getting any appreciable change in pressure. This fact seems to demonstrate that the vagus center is not dependent on afferent impulses coming in over the dorsal root fibers since, when all the afferent impulses, except those over the vagi, had been prevented from reaching it, there was little change in blood pressure.

Stewart, Guthrie, Burns and Pike (5) have shown that after a total loss of function of the cerebral centers resulting from the temporary ligation of the head arteries there is a gradual return of function following the restoration of the circulation and that this return occurs in a fairly definite order. In a second publication, Stewart and Pike (6) reported a more detailed study of the time relations of the resuscitation processes in the most important of the bulbar centers, namely, the respiratory, vasomotor and cardio-inhibitor. Since by the production of complete cerebral anemia all the reflexes of the anemic region are temporarily abolished, advantage was taken of the opportunity afforded for the study of the relation between the return of such functions as respiration, or cardiac inhibition, and the opening up of the reflex pathways to or through their centers. In regard to the respiratory mechanism these authors found it was, of the three, the most automatic and the least dependent upon reflex stimulation for the origination of its discharge. The cardio-inhibitory center was the least automatic and is the most dependent upon such impulses while the vasomotor mechanism occupied an intermediary position between the other two mechanisms. The cardio-inhibitory center regains its power of being affected by reflex stimuli sooner than it does its tone.

Stewart (7) has also shown that in dogs the rate of the heart when isolated from its extrinsic nerves by cerebral anemia is relatively constant when the external conditions (temperature and pressure of the blood) are kept constant.

The rôle played by the accelerators is of course extremely important in a consideration of the cardio-vascular system. That they are purely efferent nerves has been generally believed, but whether they are dependent on afferent impulses or whether they work automatically has not been definitely determined. Tschirjew, Stricker and Wagner have witnessed, after section of the vagi, a decreased pulse rate on

excision of the inferior cervical and the first thoracic ganglia. It is also claimed that after section of the accelerators, inhibition by excitation of the vagus can be induced with great ease. Timofeew has kept animals for some time after section of the accelerators and noticed in them a gradual decrease in pulse rate. This fact evidently points to a tonic action maintained by the accelerators but it is held in check by the greater tonicity of the vagus center. It affords evidence also that the accelerators hold the vagus mechanism in check.

Hunt and others have suggested that the tone maintained by the accelerator nerves is on blood pressure, since extirpation of the accelerators or the stellate ganglia is followed by a fall in blood pressure, in some cases amounting to 50 per cent.

The question as to whether reflex cardiac acceleration may occur independent of the cardio-inhibitory center is one which has been fairly well investigated but on which there still exist many contradictory statements. There are two possible conditions under which reflex cardiac acceleration may occur: *1*, through a depression of the vagus action; or *2*, through a stimulation of the accelerator center. A great many investigators seem to favor the former view. Roy and Adami (8) conclude that the acceleration of the mammalian heart may occur only through the vagus center. Hunt confirms this view.

In opposition to this view are those who maintain that a reflex cardiac acceleration may occur independent of the cardio-inhibitory center. Among these are Francois-Franck (9) who states that although the vagus center predominates over accelerator action under normal circumstances yet the reverse may be true under experimental conditions. Von Cyon (10) and Bayliss (11) also accept the view that reflex cardiac acceleration may take place through a stimulation of the accelerator center. The latter author has stimulated the depressor after section of both vagi and obtained an accelerated heart. Hering (12) observed in rabbits in which the last cervical and first thoracic sympathetic ganglia had been extirpated, that cardiac acceleration following muscular exercise was very much more reduced after than before the operation. That the second view is more tenable has also been confirmed by Hooker (13).

Gasser and Meek (14) claim that the acceleration of the heart at the beginning of voluntary exercise in the normal animal is chiefly due to the decrease in the tone of the cardio-inhibitory center. Their work leads them to believe that the accelerators are a factor of safety and that, in exercise, their action is superimposed on that of the vagi only in times of great need. Aside from this these authors claim that their chief function is to maintain the level of the resting pulse.

Experimental procedure. All the experiments were done on cats. The laboratory procedure was ether anesthesia and tracheotomy. Blood pressure was recorded by means of a mercury manometer connected to the left carotid artery. Records were made on a Hürthle kymograph. The time was recorded in seconds by a Jacquet chronograph. A signal magnet served as the base line for blood pressure. At all times a sufficient amount of ether was given to abolish all sense of pain in the animals experimented on. The depth of anesthesia was judged by the presence or absence of the corneal reflex. The heart rate was computed in beats per minute. In every case a control tracing of blood pressure and heart rate was taken before any other procedure was attempted.

Atropine was administered either directly intravenously or indirectly by stomach tube. In the former case the femoral vein was dissected out and a cannula was inserted into it. Atropine was injected by means of a hypodermic syringe, the needle of which was plunged into a rubber tube attached to the cannula. Backflow was prevented by a clamp on the free end of the rubber tube. In the latter case, a rubber tube was inserted into the esophagus until it reached the stomach. The drug was injected by plunging a hypodermic needle directly into the rubber tube.

The solution of atropine was made up by weighing out a definite amount of atropine sulphate and dissolving it in a definite amount of one-seventh molecular solution of sodium chloride. In this way it was known how many milligrams each cubic centimeter of solution contained, and the dose was injected accordingly. The solution kept very well in a stoppered bottle at room temperature for several days. The dose injected was about 2 mgm. contained in 1 cc. of saline solution. This amount was, however, not strictly adhered to, since the animals varied from half-grown to full-grown cats.

I. Division of both vagi with the accelerators intact. In animals such as the rabbit in which the depressor fibers of the vagus of most individuals run in a separate strand, the effect of the division of the vagi may be observed independently of the effect of the section of the depressor fibers. In the cat and the dog, this separation of the two kinds of fibers cannot be made so easily. As has been indicated, section of the vagi may be done with or without the concomitant division of the depressor according as the rabbit or some other mammal is used for the experiment. In cats, which were used throughout these experiments, bilateral vagotomy is followed usually by an increase of heart rate and blood pressure The effect varies somewhat with the general systemic blood pressure at the time the section is made and with the general

depth of anesthesia. If for any reason the heart rate is abnormally high, there is no increase on division of the vagi.

In some of the cats used, separate depressor fibers were found, but the number was so small that even in those animals in which the separate depressor fibers were found the section included these fibers. It might be added here that very often a great deal more trouble was encountered in animals with separate depressor nerves than in those in which these fibers run in the vagus. Not only was this my experience but also that of Doctor Coombs who was doing experiments in this laboratory at the time this work was in progress.

Both vagi, after being carefully dissected out, were exposed for a distance of about 2 cm. and then loose ligatures were passed around them. The section of both nerves was made practically simultaneously. The results of double vagotomy are found in the table below, which gives an average of about fifteen such experiments.

TABLE 1

	RATE	PRESSURE
Before division of both vagi	212	136
After division of both vagi	229	151
Percentage change	8	11

In practically every case studied, bilateral vagotomy was followed immediately by a rise of systemic pressure and an increased heart rate. In some instances the rise in pressure was preceded by a slight fall which was probably due to a stimulation of the efferent fibers of the nerves during the process of lifting them or by the very act of cutting them. This initial fall was very slight, however, and was immediately followed by the usual rise. It will be observed on consulting the table that there is a change both in heart rate and blood pressure.

In some animals section of both vagi was frequently followed by a great impairment of respiration. The animals had great difficulty in respiration, and very often artificial respiration had to be given for several minutes. In some animals the blood pressure fluctuated greatly for some time after bilateral vagotomy, but after a short time the usual rise in pressure and acceleration of the heart was obtained, and this was maintained fairly uniformly until the end of the experiment.

II. Division of both vagi after previous removal of the stellate ganglia. The method used in the dissection for the stellate

ganglia was suggested by Professor Pike. It is safer than any of the other methods tried since the chances of puncturing the pleura and producing a pneumothorax are much lessened by this method.

The pectoralis muscles were dissected away. The initial incision was made in the midline of the sternum, thus avoiding most of the large blood vessels. After this dissection, the ribs and the intercostal muscles were exposed. The next group of muscle to be dissected away was the chief muscles of the neck which included the cleidomastoid, sternohyoid, sternomastoideus, sternothyroideus and the scalenus. The trachea and the esophagus were thus exposed. With a pair of pointed forceps around which a small piece of cotton was wound, the highway of the esophagus was followed until a point where merely moving the esophagus slightly aside revealed the inside of the thorax. The stellate ganglia lie between the first and second ribs, and merely pushing the muscles aside reveals them. With a fine pair of pointed forceps the ganglion was raised and then removed with a fine pair of scissors.

Removal of both stellate ganglia is immediately followed by a considerable fall of blood pressure, at times more than 50 per cent. The heart rate slows and neither pressure nor rate increases. If both vagi are sectioned following the removal of the stellates there is a gradual rise of blood pressure which, however, never reaches the pressure which was maintained before the stellate ganglia were removed. There is also a striking increase in heart rate. It would seem as if section of the vagi allows the heart to escape from the tone which these nerves maintain over it. However, the rate which was maintained at the beginning of the experiment is never regained, as reference to table 2 will show.

It will be seen, therefore, that the removal of the stellate ganglia is followed by an immediate large fall in pressure, to a level which is maintained throughout the experiment provided no other lesions are made. The heart rate also decreases and this decrease is constant under the same conditions. But if bilateral vagotomy is now done, the pressure goes up somewhat but never reaches the normal level. The same is true also of the heart rate. In other words, it would seem as if blood pressure and heart rate are maintained by the stellate ganglia and the accelerator nerves while the vagi are concerned with keeping up a slight tonic action on blood pressure, but that their main function is to keep the rate of the heart down so that it is not overworked.

Hunt (15) has shown that the maintenance of a certain degree of blood pressure seems in some way to be connected with the stellate ganglia as is shown by what happens when they are removed, for then

both the rate and the pressure fall. Wickwire (16) has shown that the removal of the accelerators alone results in a moderate decrease in heart rate and a great fall in pressure.

Reference to the following table will bring out the points mentioned above more clearly.

III. Atropine: Vagi and accelerators intact. Atropine is an alkaloid which paralyzes the terminations of a number of nerves, more especially of those of autonomic, as distinguished from sympathetic, origin that supply the involuntary muscles, secretory glands and the heart. By virtue of this property, it paralyzes the inhibitory terminations of the vagus in the heart and stimulation of this nerve therefore causes no change or at least, no decrease of the pulse after its administration (17). Small quantities of atropine have no further action on the heart than the paralysis of the inhibitory nerve endings. The terminations of the accelerator nerve endings are not affected. The heart

TABLE 2

	RATE	PRESSURE
Before removal of stellate ganglia	173	147
After removal of stellate ganglia	149	92
After division of both vagi	160	105
Percentage change when the stellate ganglia are removed first	8	29

therefore after the administration of this drug is in a position as if the efferent fibers of the vagi had been cut in the neck. This results in a marked quickening of the heart.

In these experiments atropine was at first injected intravenously. In each case the heart was markedly accelerated. At the beginning there was always a decreased pressure which is probably due to the action of the drug on the heart musculature itself. In later experiments atropine was given by stomach tube so that its absorption into the blood stream was much slower than when given directly intravenously. In such cases besides getting the acceleration of the heart, a slight rise of blood pressure was also obtained. In other words, administration of atropine in small quantities into the blood stream has the same effect as bilateral vagotomy except that the afferent pathway of the vagi is open. Table 3 will show this fact more clearly.

Schiff (18) about fifty years ago maintained that stimulation of the peripheral end of the vagus nerve after atropine had been administered,

always resulted in an increased heart rate. In other words, he showed that the vagus contains accelerator fibers and that after atropine was injected the inhibitory fibers were paralyzed while the accelerator fibers were not acted upon, so that stimulation of the peripheral end of the cut vagus resulted in a stimulation of the accelerator fibers with the result that the heart rate was accelerated. Arloing (19) also maintained that there were accelerator fibers in the mammalian vagus. I repeated this experiment and obtained the same results. In every case, practically, stimulation of the peripheral end of the cut vagus after atropine had been administered gave an increased heart rate.

Another point of interest which was brought out in these experiments is the fact that the accelerators are receiving afferent impulses from the vagi and that therefore there is a definite relationship between afferent

TABLE 3

	RATE	PRESSURE
When injected intravenously:		
Before injection of atropine	183	145
After injection of atropine	195	122–106–97–92–83
When injected by stomach tube:		
Before injection of atropine	210	127
After injection of atropine	216	132
Percentage change	3	4

to efferent roots in the maintenance of blood pressure. The following series of experiments will tend to bring this point out.

Atropine was administered either directly intravenously or by the stomach tube, and sufficient time was given for its absorption and action.

The vagus contains both afferent and efferent fibers but only the latter are affected, leaving the afferent pathway intact. After the lapse of a short time either the right or the left vagus was sectioned, and its central end ligated. A medium tetanizing current was then used to stimulate this central part of the nerve. If the stellate ganglia and the accelerator nerves were intact a rise of pressure was always obtained. If however the accelerators had been removed, this rise in pressure was never obtained, but a fall was the rule. In other words afferent impulses from the vagus nerves reflexly affect the central accelerator mechanism, so that this is in a measure dependent for its activity on the afferent impulses coming over the vagi.

IV. Atropine: Vagi intact but with prior excision of the stellate ganglia. In this series of experiments excision of the stellate ganglia was immediately followed by a great fall of pressure and a decreased heart rate. After the administration of atropine, either intravenously or by the stomach tube, the blood pressure rose slightly while the heart rate also increased. Administration of atropine after the removal of both stellate ganglia gives results somewhat similar to those following bilateral vagotomy after excision of the stellate ganglia. This series of experiments again illustrates the fact that the maintenance of a certain degree of blood pressure is dependent upon the accelerators, while the vagi maintain a slight tonic action on blood pressure after the removal of the accelerators or the stellate ganglia. Likewise a tonic action is maintained by the vagi on heart rate, for after their section or paralysis by atropine the heart is immediately accelerated, although the rate maintained at the beginning of the experiment is never reached, if

TABLE 4

	RATE	PRESSURE
Before excision of the stellate ganglia	202	152
After excision of the stellate ganglia	184	82
After the injection of atropine	190	115
Percentage change	6	24

the vagi are sectioned or paralyzed by atropine subsequent to the excision of both stellates.

Stimulation of the central end of the right or left vagus with the other one intact, and with atropine administered, but with the stellates removed, results in a fall of pressure, whereas with the stellates intact the usual rise of pressure is obtained. This again illustrates the fact that the central accelerator mechanism is in a measure dependent for its activity on the afferent impulses coming over the vagi.

V. Section of accelerators, vagi intact. Section of the accelerator branches from the stellate ganglia results in a fall of pressure. The heart rate however does not seem to be reduced as much in this case as when the stellate ganglia are completely removed, in which case there is a great fall in pressure and heart rate. With the accelerator branches alone sectioned, the pressure subsequently goes up slightly and with it the heart rate is somewhat accelerated. This is possibly, even probably, due to accelerator impulses reaching the heart through the cervical ganglia (20), (21).

VI. Section of accelerators after division of both vagi. Section of both vagi is followed by the usual train of events, namely, an accelerated heart and a rise of blood pressure. This increased heart rate and blood pressure is maintained if no other experimental lesions are made, but if the accelerators are sectioned subsequent to bilateral vagotomy there is a fall of pressure and a decreased heart rate. The fall of pressure due to section of the accelerators is not as great in this case as in the case where the stellate ganglia are first removed and double vagotomy done subsequently, but the change in heart rate in the first case is greater than in the second case. It would thus appear that the ultimate heart rate and the ultimate blood pressure depend considerably on whether the accelerators or the vagi are sectioned first.

TABLE 5

	RATE	PRESSURE
Before accelerators are sectioned	158	147
After accelerators are sectioned	156	95
Percentage change	2	35

TABLE 6

	RATE	PRESSURE
Before the division of both vagi	202	138
After the division of both vagi	218	152
After section of the accelerators	180	105
Percentage change	11	24

With the ultimate heart rate the opposite seems to be true, namely, there is a greater final fall in heart rate when the vagi are sectioned first and this procedure followed by section of the accelerators than when the accelerators are sectioned first and this procedure followed by double vagotomy.

VII. Section of the accelerators after administration of atropine. In practically all respects this series of experiments agrees with the results that would be obtained if the vagi had been sectioned and this procedure followed by the removal of the accelerators. The percentage changes, however, were not quite as large. Upon the administration of atropine the blood pressure rose and the heart rate was increased. Now if the accelerators are removed, blood pressure falls

while the heart rate decreases. It would seem that if the vagi are first paralyzed by atropine or sectioned, the subsequent removal of the accelerators is followed by a smaller fall of blood pressure and a slightly greater decrease in heart rate. Immediately upon the removal of the accelerators, the pressure falls; in time the pressure gradually rises, but the initial pressure and the heart rate are never reached again.

It would thus appear that when all the extrinsic cardiac nerves are cut, either the heart rate falls somewhat and blood pressure considerably or there may be a greater decrease in heart rate and only a moderate fall of blood pressure. The final result seems to depend on whether the vagi or the accelerators are removed first. Table 7 will bring this point out clearly.

VIII. Section of the vagi subsequent to ligation of the adrenals. This series of experiments was undertaken for the purpose of seeing whether adrenalin played any part in the maintenance of blood

TABLE 7

	RATE	PRESSURE
Before the administration of atropine	210	127
After the administration of atropine	216	132
After the accelerators are removed	194	105
Percentage change	10	20

pressure under the conditions given. In every case that has yet been investigated, the action of adrenalin on muscle is identical with that produced by stimulation of the sympathetic nerve supply to that tissue (22). Since the accelerators belong to the sympathetic nervous system, it was thought that there might possibly be some relationship between their influence over blood pressure and the amount of adrenalin secreted. The usual procedure in dissecting out the adrenals was to make an incision in the mid dorso-ventral line near the region of the kidney, great care being taken not to injure the diaphragm. The fat and fascia around the adrenals were carefully dissected away leaving the gland free all around. The adrenal lumbar vein which carries away the secretion of the gland was tightly ligated and then to make doubly sure a ligature was passed around the entire gland thus shutting it off completely from the vein. In no case was there a sufficiently large hemorrhage to cause any change in heart rate or blood pressure.

Immediately after ligation of the adrenals there was a small fall in pressure in every one of the series of animals studied.

The next procedure was to section both vagi. In these experiments it was desired to see whether or not the accelerators cut off from a supply of adrenalin were able to cause the usual increased blood pressure and the usual increased heart rate, which is obtained after double vagotomy under normal conditions. Immediately following double vagotomy an increased blood pressure was obtained which lasted for a few seconds only, and then the pressure began to fall. The heart rate was however slightly increased both after ligating the adrenals and after double vagotomy.

IX. Partial cerebral anemia followed by the removal of the stellate ganglia and subsequent section of the vagi. When, for any reason, the volume of blood circulating through the brain in unit time is greatly reduced, there is a cardio-vascular reaction in a direction tending to restore the normal volume conditions in the brain. Blood pressure rises markedly, and one often finds a departure from the usual

TABLE 8

	RATE	PRESSURE
Before ligation of the adrenals	210	154
After ligation of the adrenals	226	144
Immediately after double vagotomy		160
A few seconds after double vagotomy	231	135

relations expressed in Marey's law, of a slower pulse rate with a high blood pressure. The vasoconstrictor part of the response has been studied by Winkin (26) and it seems desirable to get some further evidence upon the more strictly cardiac changes under conditions of reduced blood supply to the brain, and the rôle payed by the cardiac nerves in the response of the organism to such a change of internal conditions.

A state of partial cerebral anemia was induced by the occlusion of the right carotid artery, and the right and left vertebral arteries, but allowing a small amount of collateral circulation. Ligation of these cerebral arteries and the production of a state of partial cerebral anemia is followed in some animals by a fall of arterial pressure instead of the usual rise of pressure. This response was observed in a number of animals in this series of experiments. This condition can be remedied by releasing one of the vertebrals, usually the left. In this series of experiments the stellate ganglia were removed before the production of partial

cerebral anemia. The usual fall of blood pressure and heart rate was observed following the removal of the accelerator influences on the heart. Now when the cerebral arteries were occluded, with the exception of the collateral circulation mentioned above, the pressure rose while the heart rate slightly decreased. On releasing the carotid, the pressure fell to slightly below normal. The second occlusion was also followed by an increased blood pressure, but in this case the pressure did not rise as high as in the first occlusion, while the heart rate slightly diminished. The third occlusion and every occlusion following was accompanied by a gradual decrease in blood pressure and heart rate.

Section of the vagi during the period of partial cerebral anemia is followed by a great increase in blood pressure and heart rate as compared with blood pressure and heart rate when the vagi were still intact. Especially was there an increase in heart rate as the table below will show. Occlusion of the cerebral arteries is now followed by a pressure and rate much greater than in the case when the vagi were intact. The second and subsequent occlusions result in a rise of pressure which is much greater than in the previous case when the vagi were intact. In the case of the heart there is also an increase over the rate present when the vagi were intact, but the rate is not as high as the rate observed at the beginning of the experiment.

TABLE 9

	RATE	PRESSURE
Normal	225	130
Stellates removed	169	94
1st occlusion	183	155
2nd occlusion	173	141
3rd occlusion	160	132
Vagi sectioned	203	180
Normal circulation restored	193	133
1st occlusion	198	172
2nd occlusion	198	170
End of the experiment	188	115

A higher rate of the heart at the beginning of the experiment, than is ever attained after section of the accelerators and the vagi, indicates a tonic action of the accelerators. This is not consistent with the results of Gasser and Meek who deny the tonicity of the accelerator nerves.

X. Partial cerebral anemia followed by section of the vagi, stellate ganglia intact. A condition of partial cerebral anemia was induced as described above, with all the cardiac nerves intact. The usual rise of pressure and the slightly decreased heart rate are observed. The primary decrease in heart rate is usually followed later on by an increased heart rate if the condition of partial cerebral anemia is allowed to remain without any further lesion. This later acceleration might be explained by the following. Schiff showed that the accelerator fibers of the vagus resist degenerative changes longer than the inhibitory fibers. Arloing (19), who repeated Schiff's work, says there are four kinds of fibers in the vagus, one kind being the accelerator fibers, and confirms Schiff's work as to their greater resistance to degeneration. Other experiments have shown that the accelerator fibers retain their vitality longer after death of the animal than the inhibitory fibers. It is conceivable therefore that the accelerators are more resistant to anemia than the inhibitory fibers. Stewart, Guthrie, Burns and Pike (5) and Winkin (26) have also shown that there is a vasoconstriction in the splanchnic area during cerebral anemia. The rise in pressure and the accelerated heart may perhaps be attributed to the action of the vasoconstrictor mechanism and in part to the action of the accelerator fibers in the vagus.

The results of sectioning the vagi during a condition of partial anemia depend on whether the accelerators are present or not. If the accelerators are present, the pressure rises very high on partial occlusion of the cerebral arteries. On releasing the head arteries, the pressure never falls below the control pressure maintained by the animal; whereas if the accelerators have been excised, the pressure does fall below the control level. If the vagi are sectioned when the accelerators are not present, and then the cerebral arteries are occluded, the pressure does not rise very high and following the release the pressure falls below the control level. The accelerator mechanism seems to stand in the way of a falling blood pressure and saves the cells from a deficiency in the amount of blood circulating to them.

A contrast of the deportment of the heart when the vagi are sectioned during a state of partial cerebral anemia with the accelerator mechanism intact and with the accelerator mechanism removed is shown by the results of the following two experiments.

	BLOOD PRESSURE
Normal	122
Cerebral arteries partially occluded after section of the vagi with the accelerators intact	210
Normal circulation restored	125
Normal	130
Cerebral arteries partially occluded after section of the vagi and removal of the acelerators	140
Normal circulation restored	105

Section of the vagi during a state of partial cerebral anemia with the accelerators intact is followed by a great increase both in blood pressure and the heart rate. On releasing the cartoid the pressure and heart rate fall to a little above that maintained at the beginning of the experiment.

TABLE 10

	RATE	PRESSURE
Normal	197	128
After occlusion	191	197
Vagi sectioned	214	207
1st occlusion	217	207
2nd occlusion	212	206
3rd occlusion	212	207
4th occlusion	210	206
Normal circulation restored	200	135

XI. Partial cerebral anemia followed by section of the vagi and subsequent removal of the stellate ganglia. In this case, section of the vagi is followed by the increased pressure and heart rate described in the preceding table. Now if the stellates are removed the pressure falls very much below normal, while the heart rate also decreases. If a state of partial cerebral anemia is now induced by occluding the cerebral arteries, leaving a slight collateral circulation by way of the left vertebral artery, the pressure increases, while the heart rate remains about the same. The second and subsequent occlusions raise the pressure to about the same degree as the first occlusion, and the heart rate remains constant. It would seem as if the removal of the stellate ganglia had removed the mechanism by means of which a high blood pressure can be obtained, when it is to the best interest of the organism to have a higher pressure than is present. The absence of

this mechanism may then be injurious to the best interest of the organism when a high blood pressure and an accelerated heart rate are necessary.

XII. Section of the vagi during complete cerebral anemia. In all the experiments previous to this, the vagi were sectioned either when the entire circulation was intact or during a state of partial cerebral anemia when a certain amount of collateral circulation to the head was still open. In each case a rise of blood pressure and an increased heart was observed. Whether this effect was due to central or peripheral forces could not be determined under these conditions, and so the same experiment was repeated with complete cerebral anemia. The cerebral anemia was considered complete when all voluntary and involuntary activities had ceased. When the hind limbs became quiet, and the

TABLE 11

	RATE	PRESSURE
Normal	184	130
After occlusion	168	154
Vagi sectioned	206	197
1st occlusion	206	196
2nd occlusion	202	195
3rd occlusion	204	197
Stellates removed	184	94
1st occlusion	184	122
2nd occlusion	184	122
3rd occlusion	186	122

blood pressure began to fall, complete cerebral anemia was present. If the vagi are sectioned during complete cerebral anemia, there is absolutely no change in blood pressure or heart rate as compared to that present before the section was made. If a state of cerebral anemia however, is not present, as in the case when the circulation to the head is not interfered with at all, section of the vagi is followed by a rise of blood pressure and an increased heart rate.

This experiment then shows that the rise in blood pressure and increased heart rate, which are observed on sectioning the vagi, are a central effect and are not due to any peripheral activity for when the entire brain is made functionless by depriving it of its normal blood supply, the reactions that normally occur when the blood supply to it is intact, such as an increased blood pressure and heart rate, are abolished.

Discussion of results. At the beginning of this paper it was stated that the object of these experiments was to determine whether there was any relation of afferent to efferent roots in the maintenance of blood pressure or whether this function was entirely automatic. The central respiratory nervous mechanism and the central cardio-vascular mechanism function so nearly alike in this respect that it would be well to state here the views of Pike and Coombs (23) in regard to the nervous control of respiration. These authors in a brief summary of the organization of the nervous mechanism of respiration conclude as follows. "While it is undoubtedly true that the respiratory center may act automatically under conditions which preclude the effect of afferent impulses, it is our opinion that its normal activity is not wholly automatic but partly reflex." In various publications the same authors have shown that afferent impulses reach the respiratory center in higher mammals from the lungs and the respiratory epithelium generally, parts of the alimentary epithelium, the pleura, the muscles of the thoracic

TABLE 12

	RATE	PRESSURE
Normal	168	150
Complete cerebral anemia	160	200
Vagi sectioned	160	200

walls, the diaphragm and the abdominal muscles. If the vagi are sectioned the rate of respiration is slowed for the reason that afferent impulses coming over the vagi, which are normally summed with the carbon dioxide in the blood to produce an excitation of the cells in the medulla, are no longer present, and the excitation of the central cells is now dependent in large part upon the carbon dioxide only. If now the dorsal roots of the spinal nerves are sectioned, there is a further fall in the respiratory rate, due to a greater loss of afferent impulses to the central cells in the medulla. The cells in the medulla now become wholly dependent upon the carbon dioxide of the blood.

Briefly stated, then, the respiratory center normally receives afferent impulses from certain sensory fields. If these afferent impulses are prevented from reaching the central cells in the medulla by sectioning the vagi and the dorsal roots of the spinal nerves, respiration does not cease but continues in a modified form. The same statement can be made in regard to the central cardio-vascular mechanism. If all the

cardiac nerves are sectioned, the blood pressure does not drop to zero, but it is maintained at a certain level which is not the same as in the same animal with the cardiac nerves intact.

The nervous centers concerned with the regulation of the cardio-vascular mechanism are situated in the medulla and have a wide relation with afferent nerve fibers from all parts of the body including the heart itself. To the heart go efferent fibers which carry impulses from the central mechanism which increases or decreases its rate, and to the blood vessels go fibers which may change their caliber and thus change blood pressure. Sherrington and Wickwire have shown that section of all the dorsal spinal roots has no effect on blood pressure, and so we may concern ourselves with the changes brought about by sectioning the cardiac nerves themselves.

The problem of the relation of afferent impulses to the activity of the central cardio-vascular nervous mechanism may be divided into two parts. *1*, The determination of whether or not blood pressure may persist after section of all the afferent nerves; and *2*, the character of the response to changes at the periphery. There is little doubt that the respiratory mechanism in the medulla may function in the absence of all afferent nervous impulses. But it is equally clear that under these conditions no modifications of the respiration can be brought about by peripheral changes which cannot affect the central mechanism directly or indirectly through the blood. It is possible, also, that blood pressure may be maintained in the absence of all afferent nervous impulses, but the absence in such a steady uniform condition of any characteristic modification such as changes in the rate of respiration, would mask any noticeable manifestations of the effect of elimination of the afferent nervous impulses. One would expect, however, a failure of the usual responses to peripheral changes.

That a definite relation between afferent and efferent roots exists in the maintenance of blood pressure has been shown in an earlier part of this paper. If atropine is injected, thus paralyzing the efferent fibers of the vagi, and then the central end of the cut vagus is stimulated, a definite rise of blood pressure is obtained if the stellate ganglia and the accelerator nerves are intact; but a fall of pressure is the rule if the latter are removed. In other words, afferent impulses over the vagi reflexly affect the central accelerator mechanism.

That blood pressure does persist after section of all the afferent nerves has already been shown. In this respect the cardio-vascular system may be compared to the respiratory system, since respiration will go on

even after all the afferent nerves are sectioned, but in each case we get a modified type of respiratory or cardio-vascular response. This indicates that a certain amount of automatism is present in both these systems, but does not preclude the presence of the relation of afferent to efferent roots also.

As to the character of the responses at the periphery after the elimination of the afferent cardiac nerves, experiments indicate that there is a failure of the usual responses at the periphery. If the cerebral arteries are partially occluded when the vagi are intact, we get a rise of pressure not very much above the normal pressure while the heart rate decreases somewhat. If now the vagi are sectioned and afferent impulses from the heart can no longer reach the central cells in the medulla, a state of partial cerebral anemia results in a great rise of blood pressure and an increased heart rate. Wickwire (16) in a study of the reciprocal reactions of the cardio-vascular system has shown that when the vagi are intact compression of the abdominal aorta is followed by a compensatory response of heart rate to changing blood pressure, the magnitude of the response varying with the depth of anesthesia. The carotid blood pressure increases while the heart rate decreases. If the same experiment is carried out under similar conditions but with bilateral vagotomy, the compensatory response of a lower heart rate to increased blood pressure remains, but the degree of compensation is much less than before division of these nerves. Yates (24) has also found that if the medulla is intact, stimulation of the brachial plexus results in a rise of blood pressure. If the medulla is eliminated by producing a state of cerebral anemia the stimulation of the brachial plexus produces no rise of blood pressure. It seems safe to conclude that the response at the periphery seems to depend on whether afferent impulses can reach the central cardio-vascular mechanism. If these afferent impulses are prevented from reaching the medulla by sectioning the afferent nerves or by the production of a state of cerebral anemia the usual response at the periphery changes or fails entirely.

It will be observed on consulting table 1 that if the vagi are sectioned there is an appreciable change in both rate and blood pressure. If afferent impulses, however, can reach the central mechanism as in the case where atropine is injected, the change in rate and pressure is not so large as is shown by table 3. It would seem that afferent impulses from the heart over the vagi are necessary to keep the rate and pressure down to a normal level so that the cardio-vascular system is prevented from maintaining too high a rate and pressure. In the case where the

vagi are sectioned after both stellates are removed we also notice a greater fall in both rate and pressure than when, instead of sectioning the vagi, atropine is injected. In the latter case the afferent path of the vagus is still open and as a result of afferent impulses reaching the medulla the rate and pressure do not fall as markedly as when afferent impulses are prevented from reaching the medulla. A comparison of tables 2 and 4 will bring this point out.

Again, if the accelerators are sectioned after division of both vagi we find a greater fall in both heart rate and blood pressure than when the accelerators are removed after atropine is injected. This again seems to point to the conclusion that afferent impulses over the vagi tend to keep the central cells in the medulla in check and brings out the relation of afferent to efferent roots in the maintenance of blood pressure. A comparison of tables 6 and 7 will bring this point out.

There is no evidence from these experiments tending to show that afferent impulses over the vagi tend to inhibit the accelerator mechanism after the administration of atropine.

These experiments have also brought out some points on the function of the cardiac nerves. If the vagi are sectioned after the removal of the stellate ganglia, the heart rate and blood pressure increase considerably. The normal action of the vagus then is to maintain a tonic action over blood pressure and heart rate. This fact is brought out still more strikingly in later experiments. If the stellate ganglia are removed and then the cerebral arteries are partially occluded, with the vagus intact, a rise of pressure is obtained as well as an increase in heart rate. The second occlusion, however, results in a rise of pressure which is not as large as the first, while the heart rate also decreases. The third occlusion results in a rise of pressure which is still lower than in the second case, while the heart rate also decreases. Every occlusion after this one is followed by a still smaller rise of pressure and a decreased heart rate. These results indicate that the vagi are concerned with keeping the blood pressure and heart rate down to or sometimes below a level which is to the best interest of the organism. The vagi seem to stand in the way of a rising tide of pressure, and this obstruction may be to the disinterest of the organism. Haldane has pointed out that in the case of the respiratory system the vagi may sometimes act to the disinterest of the organism. The same statement may be made in the case of the cardio-vascular system for sometimes the vagi may stand in the way of a rising pressure when it is to the best interests of the organism to have a high pressure, as in cerebral anemia.

The results obtained on section of the vagi during a state of partial cerebral anemia seem to depend on whether the accelerators are intact or whether they have been previously excised. If the accelerators are intact, section of the vagi is followed by a very high blood pressure. When the normal circulation is restored, the pressure does not, in general, fall below the level maintained at the beginning of the experiment. If, however, the accelerators have been excised preliminary to section of the vagi, partial occlusion of the cerebral arteries results in a rise of pressure which is not very much above the pressure maintained at the beginning of the experiment. Upon restoring the normal circulation, the pressure falls very much below that maintained at the beginning of the experiment. These experiments seem to show that the accelerators are in some way concerned with the maintenance of blood pressure. That they respond to the emergency of a falling pressure is well shown, for in the case where the accelerators are intact the pressure does not fall below normal on restoring the normal circulation. In their absence, however, the pressure does fall below the pressure maintained at the beginning of the experiment when the cerebral circulation is restored.

With the loss of the accelerator mechanism the cardio-vascular mechanism loses an efficient protection against a falling pressure, which would act to the disinterest of the organism. An increased pressure is then due to the accelerator mechanism which works to the best interests of the organism.

The responses of the cardio-vascular system seem to be guided by two antagonistic mechanisms, a cardio-inhibitory nervous mechanism and a cardio-accelerator nervous mechanism, both of which through the algebraic summation of their effects act in the best interests of the organism under ordinary conditions. The first mechanism is concerned with preventing the heart from overwork, and in giving the heart musculature a sufficient period of rest for its metabolic repair. The second mechanism is intimately concerned with the maintenance of a certain level of blood pressure which is to the best interests of the organism.

Friedenthal (25) has shown that animals in which the cardiac nerves have been sectioned exhibit no discomfort when at rest, but are unable to undergo any considerable exertion. This illustrates the tone, accelerator as well as inhibitory, which these nerves maintain over the heart.

The vagus, although the inhibitory nerve to the heart, has been shown in these experiments to contain accelerator fibers. If atropine is

injected, the inhibitory fibers are paralyzed, whereas the accelerator fibers are not affected. If the peripheral end of the cut vagus is now stimulated, the heart rate is accelerated instead of being inhibited. The presence of these fibers in the vagus may also explain in part the increased heart rate which is obtained after a preliminary decrease when a state of partial cerebral anemia is brought about. This is due to a greater resistance of the accelerator fibers to anemia than the inhibitory fibers, hence they will retain their activity after the inhibitory fibers have been made functionless by anemia.

In the experiments in which the adrenals had been ligated preliminary to section of the vagi, the usual result of section of the vagi under these conditions was first a small rise of blood pressure followed by a fall in each case. The heart rate was, however, slightly increased both after ligation of the adrenals and after double vagotomy. What this factor was which caused the accelerated heart after these procedures was not determined.

I wish to express my sincere thanks to Professor Pike for the suggestion of this problem and for his keen interest shown while these experiments were going on.

CONCLUSIONS

1. While the cardio-vascular system may undoubtedly act automatically under conditions which preclude the effect of afferent impulses its normal activity and particularly its response to changing conditions in the organism is not wholly automatic but partly reflex.

2. Afferent impulses over the vagi reflexly affect the central accelerator nervous mechanism.

3. When the cardiac nerves are sectioned, the ultimate heart rate and the ultimate blood pressure depend on whether the vagi or the accelerators have been sectioned first.

4. The accelerators respond to the emergency of a falling blood pressure.

5. The accelerator nerves seem to maintain a tonic action over the heart.

6. The accelerators seem in some way to be concerned with the maintenance of blood pressure.

7. There is a cardio-inhibitory and a cardio-accelerator mechanism, both working, under normal conditions, to the best interests of the organism.

8. There are accelerator fibers in the vagus.

9. Adrenalin seems to be concerned with the rise of blood pressure on section of the vagi under the conditions of these experiments.

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SOME EFFECTS OF VARIOUS ENVIRONMENTAL TEMPERATURES UPON THE BLOOD OF DOGS[1]

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Our object in undertaking this research was to acquire a more intimate knowledge of the specific effects of high environmental temperatures upon certain individual organs and tissues of the body, so that general effects as observed among furnace workers could be interpreted more accurately than has heretofore been possible.

Unfortunately, there are no animals in common use in the laboratory which are wholly suitable for such a study. Of the domestic animals the horse is perhaps to be preferred because of the greater similarity of its heat-controlling apparatus to that of man, but inasmuch as facilities for handling animals of this size were not available we were compelled to consider the smaller animals. Of these, dogs were selected as being the most tractable and, all things considered, the best adapted for the work which we planned to do. In making this selection it was frankly recognized that the dogs have developed a method of cooling the body by the evaporation of water which is strikingly different from that which obtains in the horse and man. However, in spite of this dissimilarity and its possible effects upon the gases of the blood, our results seem to indicate that any differences are quantitative and that the qualitative changes in gas content which result from exposure to high temperatures are the same for the two forms. Aside from this theoretical objection the dogs proved to be as nearly ideal as could be hoped for.

We have centered our attention on a single tissue—the blood. This tissue was chosen because of the rapidity and accuracy with which it reflects changes taking place throughout the organism, and because it is the only tissue which may be sampled at intervals and still leave the animal in what may be presumed to be an approximately normal condition. The blood was analyzed for oxygen content and capacity, carbon dioxide content and capacity, sugar and total solids,

[1] Approved for publication by the Surgeon General.

all of which were determined as a matter of routine. In a few cases the iron of the blood was determined as a control on the oxygen capacity. In a second series the hydrogen-ion concentration and the carbon dioxide content of the plasma were determined as routine, while an occasional determination of the lactic acid in the blood was made.

In presenting the data which we have accumulated, we are aware that various investigators, from the time of Claude Bernard on, have reported observations on the blood of animals which were exposed to high temperatures, but it seems to us that these results are not only fragmentary but in some cases of such doubtful accuracy that a clear-cut interpretation is practically impossible. The inaccuracies, where they exist, are due not only to faulty chemical technic but frequently to an abnormal condition of the animals. For example, many of them were subjected to anesthesia, which disturbs not only the heat-regulating mechanism but also the general metabolism of the subject. Furthermore, these observations were made at various times upon various species of animals, while in the present research we have endeavored to correlate as many data and data of as many different types as is practical on a given series of individuals of a single species.

In addition to the previously mentioned observations on the blood, we have recorded the rectal temperature and the body weight whenever a sample of blood was drawn, and in certain of the experiments even more frequently.

Methods: I. *Chemical.* *a. The blood gases.* The carbon dioxide content and capacity of the blood were determined by the methods of Van Slyke (1). The oxygen content and capacity were determined by the method of Van Slyke and Stadie (2), but we found it necessary to add three or four drops more of the potassium ferricyanide solution than is recommended by these authors, possibly because our ferricyanide was the product of a different manufacturer.

All of the results reported were obtained with the old form of the Van Slyke apparatus, having the short stem. This apparatus was checked against the newer form having the longer stem and the water jacket and we did not feel that the differences in the results were sufficient to warrant the extra time required by the latter apparatus; for this would have necessitated an additional observer and, in our opinion, the differences due to personal equation would have more than offset the greater precision of the later apparatus.

b. The sugar of the blood. MacLean's method for 1 cc. of blood (3), as modified by Hastings and Hopping (4), was used throughout the work.

c. The total *iron* of the blood was occasionally determined as a means of controlling the oxygen determinations, using the method published by Brown (5). In this work we found that the colorimeter gave a more satisfactory means of comparing the colors than did the method described by the author.

d. The *lactic acid* was determined by the method which we have described elsewhere (6) except that the filtrate was extracted with ether and the determination made on the ether extract instead of on the filtrate directly. This was done upon the suggestion of Dr. Isidor Greenwald, in order to avoid the disturbing influence of the sugar of the blood. It is recognized that this method is not specific for lactic acid but since we were unable to find indications of an increase we feel that it is sufficient to justify the conclusions which we have drawn.

f. The *total solids* were determined by drying 1 cc. of blood to constant weight in an electric oven at 110°C. This was done in duplicate in silica crucibles.

g. The hydrogen-ion concentration was determined by the colorimetric method described by Cullen (7). The phosphate solutions which were used as standards of comparison were checked by means of the potentiometer.

II. *Physical. a. The temperature chamber.* The chamber in which the animals were exposed to the various environmental conditions was constructed of two layers of beaver board separated by a 4-inch air space. The chamber contained three windows, one at one end, another on one side and a third on the top. These windows were each about 2 feet square, and consisted of two sheets of glass with a 2-inch air space between them. A gas stove was placed inside at one end, under the window; at the opposite end there was a single wooden door lined with beaver board. The inside dimensions of the chamber were 4 feet in width by 7 feet each in length and height.

The method of heating, of heat control and of ventilation was that described by Hastings (8), and it has proven very satisfactory. The gas flow was controlled by a Roux regulator. A 6-inch electric fan was installed to overcome stratification and pockets in addition to the use of convection currents as recommended by Hastings. Thermometers placed in various parts of the chamber showed no stratification or variations in temperature greater than one-half of one degree Centigrade, while the temperature of the chamber as a whole did not vary more than one degree throughout the day.

b. The *relative humidity* within the chamber was determined by means of a sling psychrometer.

c. The *body temperature* was taken by an ordinary certified 1-minute clinical thermometer inserted in the rectum. The thermometer was left in place for at least $1\frac{1}{4}$ minutes.

d. The *respiratory rate* was counted by means of a Fitz pneumograph writing on a smoked drum through a Marey tambour. This technic was made necessary by the extremely high rates of respiration encountered at the higher temperatures.

III. *Biological.* All of the dogs used in these experiments were adult, short-haired mongrels, varying in weight from 10 to 15 kilograms. They were kept, when not actually in use for the experiments, in a kennel on the roof, and were maintained in a healthy condition throughout. It is needless to say that they were used exclusively for the purpose of the research here reported.

Their diet consisted of bread and cooked meat in an amount at least sufficient to maintain their weight. As a matter of fact, most of them gained in weight during the course of the experiments. They received no food during the period of 18 hours preceding each experiment. No food or water was permitted during the period of the experiments.

It was our practice to bring the dog which was to be used for an experiment to the laboratory at least half an hour before actually beginning the work, in order that he might become quiet and somewhat accustomed to conditions and to the personnel of the laboratory before the initial sample of blood was drawn. A rest period of at least two weeks was permitted each dog between experiments to allow recovery from any deleterious effects caused by the high temperature or from the hemorrhage attendant upon the experiment.

During the exposure in the heat chamber the animals were either confined in a cage or tied, so that while they had a certain amount of freedom they could not come in contact with the stove or otherwise injure themselves or the apparatus. At the lower temperatures they usually rested quietly or slept unless disturbed for purposes of observation. At 45° and 50°C. they were somewhat restive during the first few minutes of the exposure but later became quiet.

All of the blood samples were drawn from the jugular vein by venepuncture, in no case was venesection practiced.

For the convenience of the reader the results are presented in the form of graphs. These have been plotted from the means calculated from the various values obtained at the end of each interval and at each temperature. In order to obtain some idea of the mean average condition of the animals at the beginning of the experiments, the means and the mean standard deviations for all of the initial values of the several determinations were calculated. These mean initial

values we have used as a base with which to compare the effects of the experimental procedures. This was done by adding algebraically to each of the means of an experimental series an amount sufficient to make the initial value of that series equal to the mean of all the corresponding initial determinations. Exactly what was done will be made clear by reference to table 1. In this table are shown the means obtained for the initial determinations and those for a single

TABLE 1

To illustrate the method used in tabulation and the derivation of the data used in plotting the curves

	Body weight	Rectal temperature	Per 100 cc. of blood					
			CO_2 content	CO_2 capacity	O_2 content	O_2 capacity	Glucose	Total solids
Initial values, 47 observations								
	kg	*°C.*	*cc.*	*cc.*	*cc.*	*cc.*	*mgm.*	*mgm.*
Mean values		38.7	46.7	62.5	17.7	25.1	91	21.7
Mean standard deviation			±4.8	±4.9	±3.4	±3.1	±11	±2.1
Chamber temperature 20°C. Relative humidity 0.46; 12 observations								
Initial mean	14.8	38.9	45.2	67.0	18.4	25.3	92	22.2
Correction added		−0.2	1.5	−4.5	−0.7	−0.2	−1	−0.5
Modified mean		38.7	46.7	62.5	17.7	25.1	91	21.7
Mean standard deviation			±5.4	±3.3	±3.0	±3.5	±12	±2.0
2nd hour, mean	14.8	39.0	45.8	66.9	19.0	25.7	87	22.4
Modified mean		38.6	47.3	62.4	18.3	25.5	86	21.9
Mean standard deviation			±5.6	±3.3	±3.0	±3.9	±17	±1.8
4th hour, mean	14.7	38.2	46.0	67.7	18.8	25.6	85	22.8
Modified mean		38.0	47.5	63.1	18.1	25.4	84	22.3
Mean standard deviation			±5.3	±3.7	±3.1	±3.0	±16	±2.5
6th hour, mean	14.7	38.3	46.0	67.9	18.8	25.5	83	22.3
Modified mean		38.1	47.5	63.4	18.1	25.3	82	21.8
Mean standard deviation			±5.2	±3.6	±3.4	±3.4	±15	±1.9

temperature—20°. Together with the means are shown the correction which was added and the modified mean so obtained. This last was used in all cases in plotting the curves shown. (cf. Scott and Ford (9).)

The formula used in calculating the mean standard deviation was:

$$\sigma = \frac{\sqrt{\frac{\Sigma (d)^2}{N-1}}}{\sqrt{N}}$$

where σ = the mean standard deviation; d = the arithmetical difference between the individual determinations and the mean for the series, and N the number of observation.

It is planned to publish shortly the complete data obtained in this research together with additional material as a bulletin of the United States Public Health Service. It is hoped that those interested in the detailed results will make use of this publication.

TABLE 2

To show the number of observations made and the individual dogs used in the first series, embracing determinations of oxygen content and capacity, carbon dioxide content and capacity, blood sugar and total solids

CONDITIONS		OBSERVATIONS ON DOG						TOTAL OBSERVATIONS
Temperature	Mean relative humidity	E	H	J	M	P	Q	
°C.								
Initial		7	4	5	10	10	10	46
20	46	2	2	2	2	2	2	12
30	43	2	2	2	2	2	2	12
40	39	2	0	1	2	2	2	9
45	35	1	0	0	2	2	2	7
50	28	0	0	0	2	2	2	6

TABLE 3

To show the number of observations made and the individual dogs used in the second series, embracing determinations of the concentration of hydrogen ion and carbon dioxide content of the oxalated plasma

TEMPERATURE	OBSERVATIONS ON DOG				TOTAL OBSERVATIONS
	M	P	S	T	
°C.					
20	1	1	1	1	4
30	1	1	1	1	4
40	1	1	1	1	4
45	2	2	2	2	8
50	2	2	2	2	8

EXPERIMENTAL RESULTS AND DISCUSSION. The total number of experiments which were carried out together with the experimental conditions to which the animals were subjected as well as the individual dogs which served as subjects are indicated in tables 2 and 3.

I. *The effects of various environmental temperatures upon the body temperature.* The changes in the body temperature during exposure to the several conditions studied are shown graphically in figure 1.

The mean body temperature for forty-seven initial observations was 38.7°C. It was found that the body temperature fell slightly for a time and then remained at a fairly constant level for the remainder of the period of observation. This agrees with the results reported by the New York State Commission of Ventilation for men when they were subjected to similar conditions (10).

At environmental temperatures of 30° the history for the dogs was much the same as for 20° except that the fall in body temperature was not quite so marked. The New York State Commission

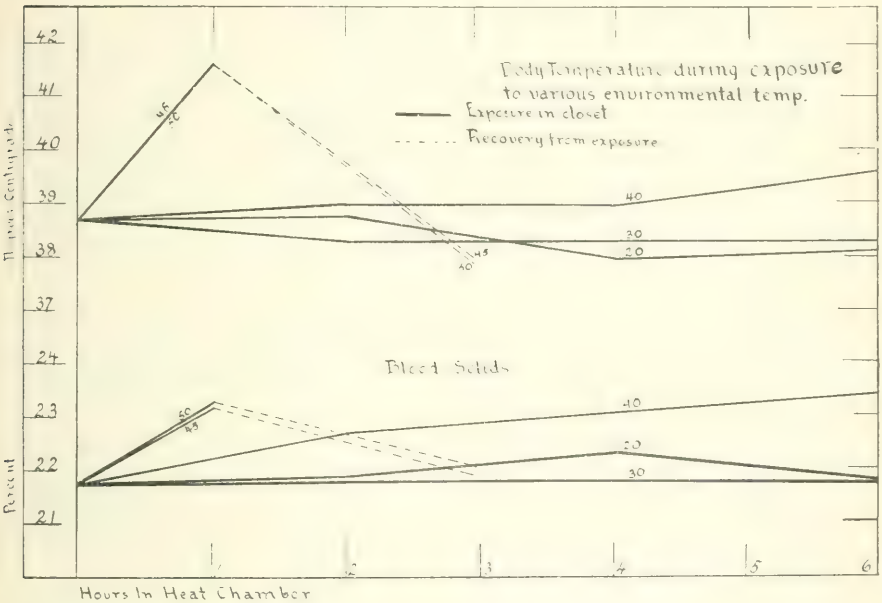
Body Temperature during exposure to various environmental temp.
Exposure in closet
Recovery from exposure
Blood Solids
Hours In Heat Chamber

Fig. 1. Curves to show the relation between various environmental temperatures and the temperature of the body and the total solids of the blood of dogs.

of Ventilation reported a slight rise of body temperature in men when they were exposed to an environmental temperature of 30°, though in their case the humidity was 80 per cent, much higher than in any of our experiments. The explanation of this behavior is not clear to us although it may possibly be related to the normal diurnal variations in the body temperature as our observations and those of the Commission were begun in the morning and continued for some time through the day. Or again it may be related to the decreased muscular activity which accompanies confinement in the chamber and to the fact that no food was taken during the course of the observations. This latter interpretation seems the more probable

in view of the fact that the diurnal curves usually shown are highest in the late afternoon and hence vary in the sense opposite to that observed by us.

A consideration of the work of Rubner (11) is of interest at this point, for he has shown that at 20°C. the dog manifests no increase in the rate of metabolism, and that this condition of unchanging metabolism persists while the environmental temperature is raised to at least 30°. He has further demonstrated that in the fasting animal the minimum energy release occurs at temperatures of from 30° to 35°C. This might be called the basal energy requirement, or the minimum energy release compatible with mammalian life.

When the temperature of the chamber is raised to 40°, the response is quite different. Here we find a *rise* of 1 degree in body temperature during the six-hour period of observation, and as a rise was apparent at the end of the first hour of exposure there was no evidence of an initial fall as observed in the two previous cases. The temperature remains constant during the middle period, the rise being confined to the first and last two-hour periods. Whether or not this is of significance we are not prepared to say.

At temperatures of 45° and 50° a very marked rise of the rectal temperature was noted, which apparently began at once. In fact this rise was so sharp that at the end of one hour the body temperature had risen to such a point that it was not deemed safe to continue the experiments at these temperatures for a longer time. On removal of the animal from the chamber the body temperature rapidly fell and two hours after removal the body temperature was subnormal, or about 36°. This subnormal temperature reminds one of the conditions in certain stages of heat stroke in man.

It will be noted that the body temperature is the same whether the animal had been exposed to an environmental temperature of 45° or 50°. This remarkable fact of a similar physiological response to conditions of different severity was noted in some of the other factors studied it being especially marked for those having to do with the carbon dioxide. The organism seems to yield with increasing rapidity as the strain impressed upon it becomes more severe, until a certain limit is reached at which point great resistance is interposed by the organism against any further change. If the strain is increased so that the organism is pushed beyond this point, it will recover with great difficulty, if at all, unless external aid is given. This same type of physiological limitation was noticed by Henderson and Haggard (12) during their work on low levels of carbon dioxide and alkali reserve induced by ether. It was also noticed by Britton in his studies on cooling. In order to lower the temperature of his

animals below what might be called the critical point he found it necessary to subject them to anesthesia until it had been passed, after which the anesthesia was no longer necessary and the body temperature continued to fall so long as the animal was exposed to an environmental temperature lower than the body temperature.

II. *The effects of various environmental temperatures upon the oxygen content and upon the amount of hemoglobin in the blood.* Considering first the oxygen capacity (hemoglobin) we find very little variation throughout the series. In fact, all of the mean values are within the limits of the mean standard deviations of the 20° series and so from a purely statistical standpoint are without significance. The direction of the slight variations which do occur fits so nicely with other circumstances, however, that one hesitates to dismiss them without further discussion. (Fig. 2.)

The changes noted at 20° and 30°, if not entirely fortuitous, may be an example of the diurnal changes studied for man and the goat by Dreyer, Bazett and Pierce (14). At temperatures of 40° and above there is a slight tendency for the oxygen capacity to increase, this tendency becoming somewhat more marked as the environmental temperature rises. This increase is paralleled by an increase in the total solids, and we have related it simply to an increase in the concentration of the blood due to the excessive evaporation of water, accompanied by an inability of the water reservoirs to supply water at a rate sufficient to meet the demand made upon them at these high temperatures.

The fact that there was no increase in the oxygen capacity during the first two-hour period of exposure to a temperature of 40° would seem to bear out this interpretation. It might fairly be assumed that at this temperature the loss would not be so rapid but that sufficient time would be permitted for equilibration and that there had not yet occurred so great a loss that the available store of water had become seriously depleted. The later rise, then, was probably due to an actual depletion of the available water below a limit where the original concentration of the blood could be maintained.

At 45° and 50° it may be presumed that while the actual quantity of water which has been lost from the tissues during the first hour of exposure is not of itself serious, the rate of loss of water from the blood is so rapid that the organism is unable to maintain the original concentration of the blood. This interpretation is further supported by the rapid return of the oxygen capacity and of the total solids to their original level during the first two hours after the animal was removed from the chamber.

A very slight fall is noted in the oxygen capacity of the animals exposed to 30°. A comparison of this fall with the mean standard deviation of the 20° series, however, indicates that it is too small to be of significance from the standpoint of statistics. Some biological considerations, on the contrary, seem to show that it may have some importance; but this evidence is not sufficient to warrant discussion at the present time.

There is a fairly marked fall in the oxygen content of the venous blood of animals exposed to a temperature of 30° as compared with

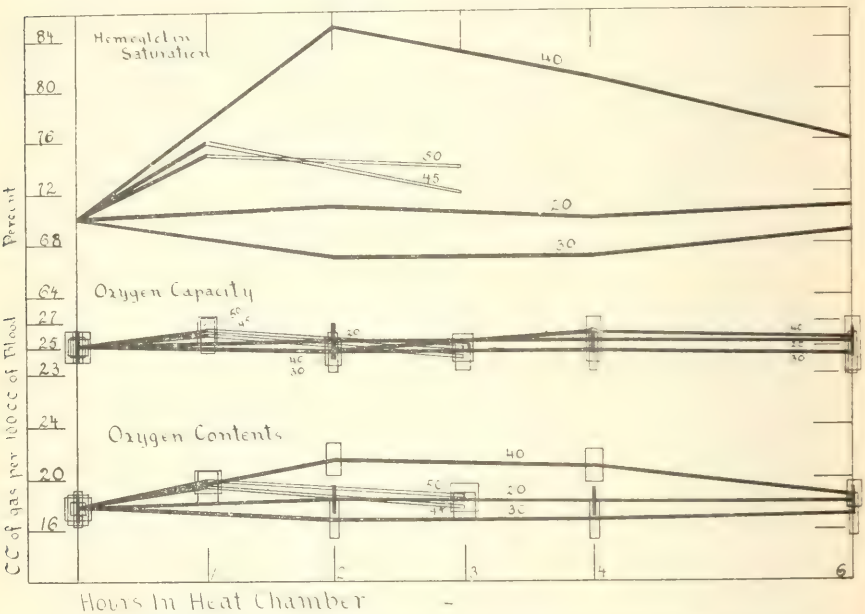

Fig. 2. Curves to show the effects of various environmental temperatures upon the oxygen capacity, oxygen content and the percentage of oxygen saturation of the venous blood of dogs.

that of animals exposed to a temperature of 20°. This drop we believe to be a reflection of the manner in which the animals respond to the two temperatures. At 30° and the humidities at which we were working, there seems to be nearly an equilibrium between the heat generated in the basal metabolism and the heat lost to the environment. (Cf. Voit (15).) The animals are quick to take advantage of this and stretch themselves out and take life easily. Hyperpnea has not yet become necessary to keep the temperature of the body down. All muscular movements, and consequently heat generation, are at a minimum. The net result is a considerably

reduced aeration of the blood, as well as a reduced circulation and consequently a reduced oxygen content, especially of the venous blood.

At 40° there is a considerable increase in the rate of respiration, and correlated with this, a rather marked increase in the oxygen content of the blood. The rate of increase in the oxygen content is slightly greater at 45° and 50°, respectively, than it is at 40°, but the increased rates are not nearly so great as might be expected when one compares them with that occurring between 30° and 40°. There are at least two factors concerned in this: First, while the rate of respiration continues to increase as the environmental temperature continues to rise it becomes progressively shallower (fig. 3), so that while the efficiency of the respiratory apparatus as a cooling mechanism may rise because of the increased passage of air over the membranes of the mouth and throat and a consequent increase in the vaporization of water, its efficiency as a means of aerating the blood does not increase in anything like the same ratio, if at all. Second, with the increased body temperature which accompanies exposure to these high environmental temperatures the rate of metabolism is increased and this would of course be reflected in a decreased oxygen content of the venous blood provided that there was not at the same time a considerable increase in the amount of oxygen carried in the arterial blood, which from the evidence just discussed, is probably not the case. The curves showing the percentage of saturation (fig. 2) are very satisfactorily explained on this hypothesis.

III. *The effects of various environmental temperatures upon the carbon dioxide content and upon the alkali reserve of the blood.* There is no change in the carbon dioxide capacity of the blood during an exposure of six hours to a temperature of 20° or of 30°, as is shown in figure 4, where it will be seen that the two curves are identical within the limits of the mean standard deviation and that both are horizontal.

When the environmental temperature is raised to 40° there is a fairly rapid fall during the first four hours and a somewhat slower fall during the remaining two hours. At 45° and 50° there is a very rapid depletion of the alkali reserve as shown by the carbon dioxide capacity, this depletion being almost identical in degree for the two temperatures. This is a very good illustration of the critical point previously mentioned in the discussion of body temperature. We believe that the changes noted result directly from the equilibration necessitated by the washing out of the carbon dioxide which, in turn, is caused by the hyperpnea due to the high temperatures—a hyperpnea which may even become dyspnea if the exposure is long continued.

The carbon dioxide content (fig. 5) gives a similar picture, except that here a slight rise is noted in the animals exposed to an environmental temperature of 30°. This probably results from the same cause as the corresponding slight depression of the oxygen content

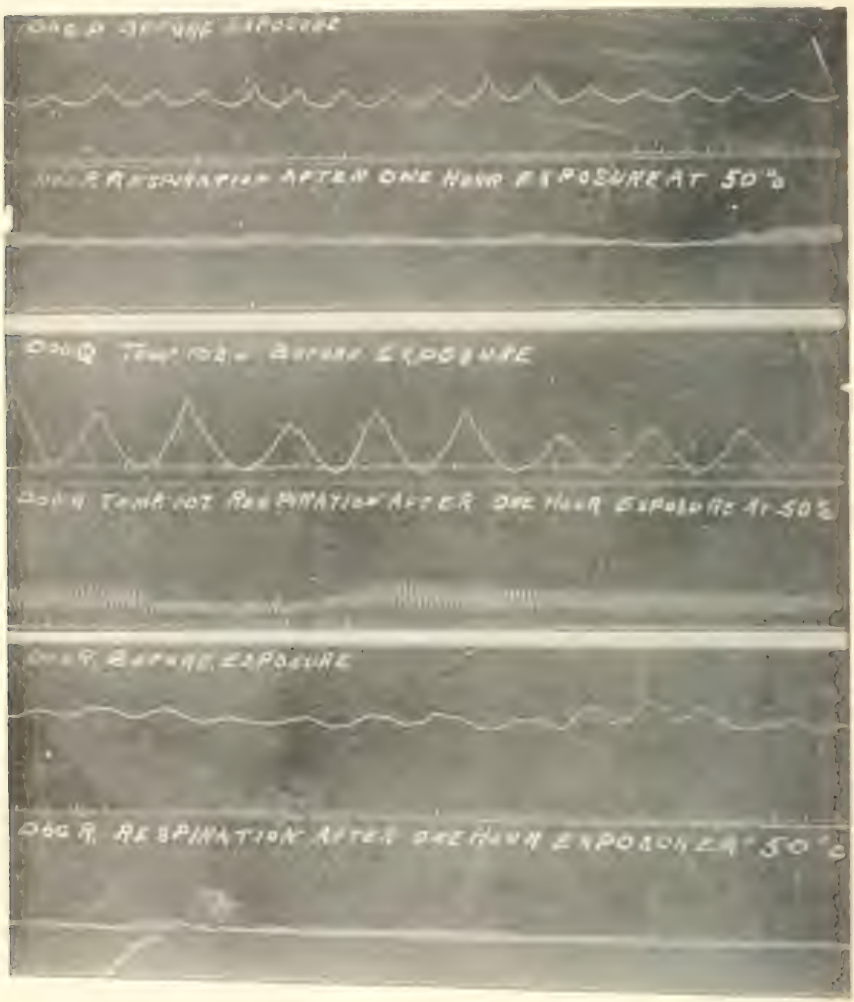

Fig. 3. Pneumographic tracings to show the relative rate and depth of respiration of dogs subjected to environmental temperatures of 20° and 50°C.

which was noted for the same condition, i.e., a small decrease in the rate of metabolism, which would be, under these conditions, at its lowest ebb as shown by Rubner and others. This decreased metab-

olism would result in a slight lowering of the rates of respiration and circulation.

IV. *The effects of various environmental temperatures upon the hydrogen-ion content of the plasma.* The hydrogen-ion concentration

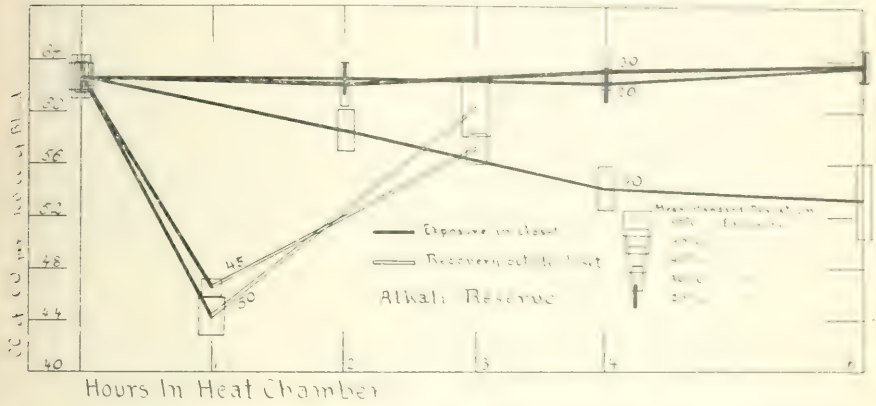

Fig. 4. Curves to show the effects of various environmental temperatures upon the alkali reserve of the blood of dogs as determined by the carbon dioxide capacity.

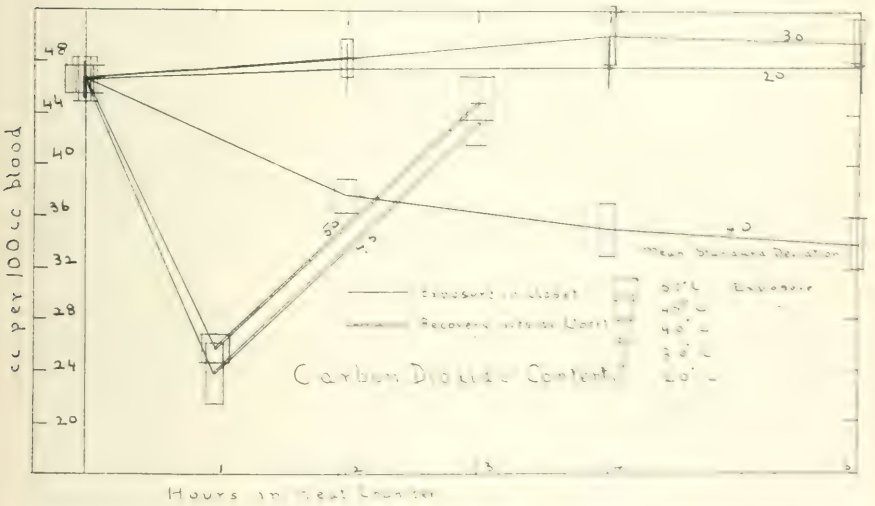

Fig. 5. Curves to show the effects of various environmental temperatures upon the carbon dioxide content of the venous blood of dogs.

expressed as pH remains constant for at least six hours when the animals are exposed to temperatures of 20° or 30° (table 4) and falls within the normal acid-base balance, or area 5 of the Van Slyke

chart (16). At 40° with an increased rate of respiration and the resultant fall in the carbon dioxide content and the alkali reserve, we find that the blood index has passed from area 5 to area 6 of the Van Slyke chart, or into the region of compensated carbon dioxide deficit. The alkali reserve likewise falls thus preventing an abnormal alkalinity. As the strain becomes greater with environmental temperatures of 45° and 50°, the pH increases from the normal of 7.55 to 7.79 and to 7.84 for the two temperatures respectively. The carbon dioxide of the plasma has dropped to 29.9 and to 26.3 volumes per cent and the plasma index has passed into area 2 or 3 of the Van Slyke chart or into the region of uncompensated carbon dioxide deficit as the result of an excessive loss of carbon dioxide. This loss of carbon

TABLE 4

To show the effect of various environmental temperatures upon the concentration of the hydrogen ion and carbon dioxide content of the plasma

TEMPERATURE OF CHAMBER	BEFORE EXPOSURES		AFTER ONE HOUR EXPOSURE		AFTER SIX HOURS EXPOSURE	
	pH	CO_2 content	pH	CO_2 content	pH	CO_2 content
°C.						
20	7.57	52.4	7.57	52.4	7.57	55.4
40	7.57	52.4	7.56	46.6	7.56	39.5
45	7.57	52.4	7.79	29.89		
50	7.57	52.4	7.83	26.3		

dioxide was induced by an increase of the respiratory rate which was evidently brought about by some stimulus other than an increased concentration of the hydrogen-ion. The same condition, also accompanied by hyperpnea, has been observed by Bazett and Haldane (17) in man when immersed in warm baths.

Kahn (18) and Barbour (19) are of the opinion that the mechanism causing the increased rate of respiration is the increased temperature of the blood, that is, it is the direct result of the increased body temperature. It is known that cellular activities in general increase, within limits, with the temperature and there is no apparent reason for excepting the respiratory center from this category. It was at first thought that the stimulus might be due to a local accumulation of hydrogen-ion within the cells of the respiratory center itself, which might occur as a part of a general tissue anoxemia depending upon the increased stability of the oxyhemoglobin at low carbon dioxide concentrations. (Cf. Bohr (20).) If such an anoxemia should in truth exist one would expect to find it indicated by an accumulation of lactic acid in the blood, but a careful search has failed to show the slightest increase

of this acid in the blood of animals exposed to high temperatures, over the amount occurring under ordinary conditions. It is difficult for us to imagine a significant acidosis occurring in the tissues without its being mirrored in the blood stream.

Our work does not permit us to accept the suggestion of Hill and Flack (21) and of Mayer (22) that fatal termination from over-heating is due to an accumulation of acid. We found a decreased rather than an increased hydrogen-ion concentration in the plasma of all of our animals which recovered from exposure to severe conditions and in none did we find any indication of increased lactic acid. From two animals, Q and R, which died shortly after removal from the chamber after having been exposed to rather severe conditions, blood was drawn only a few minutes before death. The mean values obtained were:

Oxygen content	5.9	Carbon dioxide content	30.5
Oxygen capacity	23.6	Carbon dioxide capacity	36.7

Hydrogen-ion determinations were not made, but from the Henderson nomogram (23) the pH may be presumed to have been about 7.4, which would not indicate acidosis. (Compare Henderson (24).)

Since the respiratory efforts gave every indication of the passage of at least a normal volume of air up to the time that the samples were taken we have interpreted the changes which occurred as indicating circulatory rather than respiratory failure, this having been induced by the overload thrown on the circulatory mechanism in the effort to keep the body temperature down.

V. *The relation between the concentration of the sugar in the blood and the temperature of the environment.* The changes in the concentration of the sugar in the blood (fig. 6) are rather hard to explain in a manner which is consistent for all of the conditions studied. At 20° there is a fairly uniform fall throughout the six-hour period of observation. This is in entire agreement with the observation of Scott and Hastings (25) and can hardly be related to the ingestion of food since sufficient time was allowed for this factor to become constant before the first sample was drawn. The only explanation which occurs to us is that the animals were becoming progressively quieter as the experiments proceeded and consequently were mobilizing less and less sugar. Whether such a decrease in the rate of mobilization of sugar is the direct result of lessened excitement, or whether it is due to the operation of an unknown factor which tends to equilibrate the concentration of sugar in the blood with the metabolic requirements of the organism for sugar, is a matter which we are unable to discuss at present. Aside from this factor which may be assumed, for the time being, to have been constant

throughout the research, it is to be noted that when the corresponding periods of the different series are compared a rough agreement between the concentration of the sugar in the blood and the body temperature is noted. The 45° series forms an exception to the rule, however, for at this environmental temperature the body temperature is almost identical with that which occurs when the animals are exposed to a temperature of 50°; while the concentration of sugar occupies a position midway between that found at 40° and that at 50°. This rise in the blood sugar is then not exclusively dependent upon the body temperature but seems also to be associated with the environmental temperature

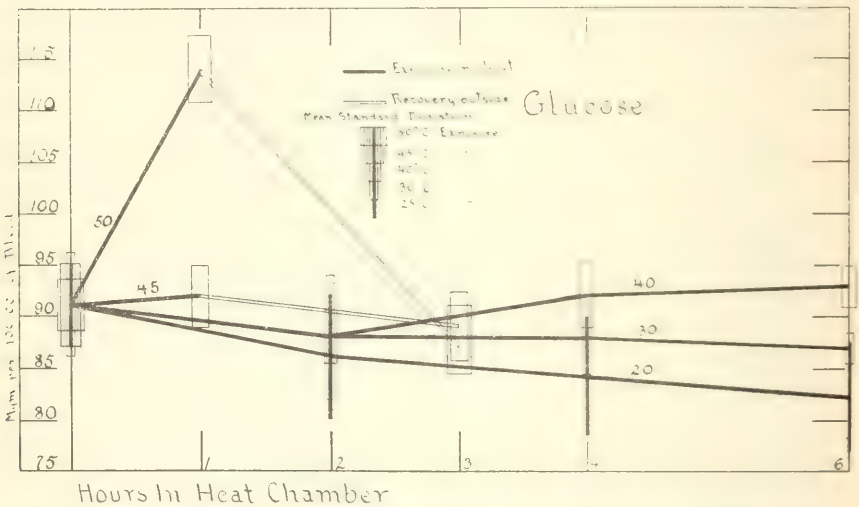

Fig. 6. Curves to show the effects of various environmental temperatures upon the concentration of glucose in the venous blood of dogs.

in a more direct manner. It is apparently not dependent upon any emotional disturbances resulting from the exposure to the higher temperatures. We hope to be able to give further evidence regarding this phase in a later paper. Lépine (26) attributes the hyperglycemia which is occasionally present in fever to an irritation of the fourth ventricle by the fever toxins. The increase in the concentration of sugar which Freund and Marchand (27) report in fever is only of such a degree that it may be accounted for by changes in the concentration of the blood. We are not, however, able to explain our results in this manner since we found the increase in the sugar to exceed that of the total solids; in one case there was an increase in the sugar of 100 per cent while at the same time the total solids increased only 25 per cent.

VI. *The relation between the total solids of the blood and the environmental temperature.* The total solids of the blood tend to increase as the environmental temperature rises and we have been unable to observe the dilution mentioned by Barbour (28) in the report of his experiments with hot and cold baths. Our results seem to be more in line with the results which he obtained in coli fever (29). There is no doubt some mechanism by which the total blood volume is regulated, and our results should be considered as indicating some interference with the activity of this mechanism. At high temperatures the rate of replacement of water cannot keep pace with the rate of its loss and a certain amount of concentration results. There is, however, a fairly large factor of safety for it is not until the concentration of the blood approaches 25 per cent that pathological symptoms occur from this cause.

SUMMARY

1. During an exposure of six hours to an environmental temperature of 20° or of 30° there was a drop in body temperature, probably due to a decrease of muscular activity. At 40° there was an increase of 1 degree in body temperature without an initial drop. At 45° and 50° the body temperature rose within an hour to such a height that it was deemed unsafe to continue the experiments at these temperatures for a longer time.

2. The oxygen capacity of the blood showed no change during an exposure to the different temperatures that cannot be accounted for by the diurnal changes in the hemoglobin or by the concentration of the blood due to the excessive evaporation of water.

3. The oxygen content of the blood remains unchanged at 20°, but shows a drop at 30° which is probably associated with the low rate of metabolism at this temperature. At 45° and 50° there is a slight increase in the oxygen content due to the increased aeration of the blood at these temperatures, but this increase is not in direct proportion to the increased passage of air over the membranes of the mouth and throat.

4. At temperatures of 20° and 30° the carbon dioxide capacity of the blood remains unchanged, while at 40° there is a sharp fall during the first four hours followed by a slower fall during the next two hours. At temperatures of 45° and 50° there is a rapid depletion of the carbon dioxide capacity from the beginning, which is almost identical for these two temperatures.

5. The carbon dioxide content follows the capacity except that at 30° there is a slight rise for the same reason that the oxygen content falls.

6. The hydrogen-ion content of the plasma remains unchanged during an exposure of the animal to temperatures of 20°, 30° and 40° for six hours, but decreases at temperatures of 45° and 50° due to the excessive pulmonary ventilation at these temperatures with the consequent washing out of the carbon dioxide and without a compensatory loss of alkali from the blood.

7. The concentration of blood sugar falls during an exposure to temperatures of 20° and 30°. This fall is probably associated with inactivity of the animal during the course of the experiment. At 40° it falls during the first two hours, to increase during the following four hours. At 45° no change was noted during an hour's exposure while at 50° there was a sharp rise during this time.

8. The blood solids at 20° and 30° showed only the usual diurnal changes, while at 40°, 45° and 50° the concentration of the blood increased with the environmental temperature, no initial drop having been observed.

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SEASONAL CHANGES IN THE GASES AND SUGAR OF THE BLOOD AND THE NITROGEN DISTRIBUTION IN THE BLOOD AND URINE OF THE ALLIGATOR

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The original purpose in investigating the blood of the alligator was to follow any changes occurring in the blood of a poikilothermic animal, when the temperature of the environment was varied. The carbon dioxide production and oxygen utilization in poikilothermic animals follow closely changes in surrounding temperatures, although there is evidence of regulatory control of metabolism within a range of temperature of from 15° to 20°C. (1). Complex changes occur in the blood of homoiothermic animals under conditions of high environmental temperatures, many of which are directly related to the mechanism of temperature control (2). Changes occurring in the blood following a change of environmental temperature ought to be more directly related to the environmental conditions in poikilothermic than in homoiothermic animals.

In the search for a suitable cold-blooded animal, the American alligator was selected because it offers the advantage of suitable size and the consequent possibility of supplying sufficiently large quantities of blood without serious disturbances due to hemorrhage.

Methods for securing the blood were worked out and preliminary analyses of the gases of the blood were made. It was found that the carbon dioxide contents were markedly lower in January and February, when the work was begun, than in the following March. In some cases there was a difference as great as twenty volumes per cent. In searching for a cause of these variations it occurred to us that they might be due to an annual rhythm, and it became necessary to temporarily discontinue the work as originally planned and to pursue the question of these seasonal variations.

The importance of seasonal changes is obvious in this connection, for it would be impossible to interpret the reaction of the animal to experimental conditions if it is at the same time responding to an unknown and uncontrolled cycle of changes, due not to its immediate environment, but to its individual or inherited history.

Extensive investigations of the nitrogen metabolism of birds have been carried out showing that uric acid is the end product of protein metabolism. Ammonia and lactic acid (3) are combined to form uric acid which also originates from amino acids (4), urea (5), ammonia (6) and from the purin bases (7).

Reptiles are generally classed with birds in their nitrogen metabolism since the excrement of the snake has been found to consist of ammonium urate (8) Among reptiles, however, there is a greater diversity in structure than among birds, and it was thought that investigation of the nitrogen end products in the alligator might add to our knowledge of the biochemistry of this class of animals.

Apparently the blood sugar of the alligator had never been determined previous to this investigation and it was felt that it should be included with the other factors studied.

Discussion of methods. *I. Biological. a. General care of animals:* We thought it advisable to maintain our animals under conditions as nearly uniform as was practical. A wooden zinc-linked tank was built, having dimensions of 5 feet × 2 feet × 2 feet. The cover was divided into three sections, the middle section being of glass, and the two end sections of heavy muslin, for ventilation. Small holes were bored through the upper portion of the tank to insure more complete ventilation. The temperature of the water in the tank was controlled by means of a hot water heating system, an electric stove being used as a source of heat. The temperature of the tank was maintained at approximately 26.5°C., a variation of 1 or 2 degrees occurring during sudden changes of weather. The water was changed once a week, and the tank cleaned. The water was heated to the proper temperature before being poured into the tank. The animals were fed twice a week during the spring and early summer, and weekly for the remainder of the period. Raw beef cut in strips was used exclusively. Each time the animals were used for experimental purposes, they were weighed and measured.

Seven alligators were kept in the tank during the fifteen months of the investigation, from January 1922 to March 1923, and at the end of the period they were to all appearances as healthy as at the beginning.

b. Method of drawing blood. Blood was drawn from the heart by means of a hypodermic needle with the precautions necessary for blood gas analysis according to the method described by Van Slyke (9). The heart beat is weak and difficult to detect by touch, and so the heart was located by means of a fluoroscopic examination. It was found to be well down in the middle of the body, between the ribs, and unprotected by the sternum. Later it was located in each animal by laying the animal on its back and examining it from the side, when a slight rise

and fall of the skin could be noted. This is essentially the same method as was used by Tiegel (10) in locating the heart of the snake. The horny scales of the epidermal skeleton are arranged in the ventral portion of the trunk in a very regular manner and it is possible by means of the transverse rows to locate the heart at the eleventh and twelfth row from the broad band just above the fore limbs. A needle two inches in length, gauge no. 20, was used. Coagulation was prevented by potassium oxalate.

Saturated oxalate solution was drawn through the needle just before using, and the needle dried by drawing air through it. To obtain the blood sample the needle is inserted through the skin, between the scales, for venous blood, about 0.5 cm. to the right of the midline of the body. When the purpose was to secure arterial blood, the needle was inserted 0.5 cm. to the left of the midline. In all cases, in searching for the heart the needle was directed parallel to the midline, being pushed gently through the tissue below the skin, very slowly, waiting for the detection of the heart beat. When the beating heart is felt at the end of the needle, a "diaphragmatic" expiration is awaited. The needle is then made to follow the heart downward. The needle is held in the final position, and the inspiration that follows pushes the heart upon the needle. By this technic the needle is inserted in the heart to approximately the same depth. Blood samples have been taken at intervals of two weeks throughout the year without the loss of a single animal.

c. Method of catheterization. Samples of urine were obtained by catheterizing the cloaca. The catheter was sterilized with 5 per cent carbolic acid, and inserted into the cloaca. The urine was collected in a small graduated cylinder, at times under oil. To prevent infection, the cloaca was washed out with 20 cc. of a saturated solution of boric acid, then washed with 20 cc. of sterilized distilled water.

In the alligator the rectum and ureters open into the cloaca. A sphincter muscle separates the rectum from the cloaca so that the cloaca acts as a bladder. The urine was collected many times, and was always found to be a limpid fluid and never contaminated with fecal matter.

II. Chemical methods. a. Blood gases. Estimation of the gases of the blood was carried out by means of the methods described by Van Slyke and Stadie (11). It was found necessary in making the oxygen determinations to add several more drops of ferricyanide than directed by Van Slyke, in order to completely liberate the oxygen of the blood; otherwise the method was followed exactly as described. The oxygen capacity of the blood was determined and the amount of hemoglobin calculated by multiplying by the factor 0.746. By a method for deter-

mining small amounts of iron, described by Brown (13), the hemoglobin was calculated from the iron and compared with that obtained by gas analysis, as a means of control. Instead of using alveolar air for the determination of the carbon dioxide capacity of the blood, samples of blood were exposed to a determined tension of 40 mm. of mercury of carbon dioxide. Ten minutes in a slowly rotating Squibb funnel were allowed for equilibration.

b. Blood sugar. The sugar of the blood was determined by the MacLean method (14), as modified by Hastings and Hopping (15).

c. Nitrogen of the blood. Total nitrogen of the blood was estimated by the Macro-Kjeldahl method (16). Urea was determined by the method described by Myers (17) and ammonia by the method also described by Myers (17), except that 3 cc. of blood were used instead of 2. In both cases the liberated ammonia was collected in N/50 H_2SO_4, and titrated against N/50 NaOH. Urea was determined also by Folin's method on the tungstic acid filtrate (18). Uric acid was determined by Benedict's new method (19), and in a few cases, by the Folin method. Creatine, creatinine and non-protein nitrogen were determined by Folin's method (18), the non-protein nitrogen also by a Macro-Kjeldahl method, titrating with N/50 alkali.

d. Urinary nitrogen. Total nitrogen of the urine was determined by the Macro-Kjeldahl method as described for blood. Urea was determined by the method of Van Slyke and Cullen (20). In carrying out the urea determination it was necessary to make the urine faintly acid to litmus before addition of urease. Ammonia was determined by the Steele modification of Folin's method (21). The ammonia content of the urine was so high that the equivalent of 0.2 cc. of urine was used for estimation of ammonia. Urease was used in tablets made by Squibb, also in powder form as made by the Arlington Chemical Company. Uric acid was determined by Benedict's latest method (21).

We wish to express here our appreciation of the help given us by Professor Benedict in carrying out this investigation.

e. Chlorides. The chlorides in the blood were determined by the method of Austin and Van Slyke (23). In the urine, the chlorides were determined by the method of Volhard-Harvey (24).

Discussion of results: *1. Health of animals.* The value of information gained by experiments carried out over a period of more than a year, upon animals kept under laboratory conditions and subjected to treatment that might be regarded as injurious, must depend largely upon evidence that the animals have not been rendered abnormal. The usual criteria by which such questions are decided are increase in weight and continued growth of the animal. Measurements of length were made over a period of six months and these showed an average increase

in length of 6 cm. for the period, or 1 cm. a month (table 1). According to measurements collected at the New York Zoological Garden, under the most favorable conditions, this animal was found to grow about a foot a year (25). Our figures are only 40 per cent of this number but, as mentioned above, the feeding of our animals was cut down considerably and was less than that as described at the Zoological Garden. Records of weight were kept and figure 1 shows that for each animal there was a gradual and steady increase. There is an exception to this statement in the earlier portion of the curve, in the case of animals 1 and 2. This was due to the difficulty experienced in finding suitable food. The flattening of the curve which is noted at the middle of the summer was due to the reduction in the amount of food at this time which was mentioned above. This reduction was necessary since the animals were becoming so large that they threatened to outgrow our facilities for handling them.

TABLE 1

Increase in length from June to December, 1922

NUMBER OF ANIMAL	INITIAL LENGTH	FINAL LENGTH	DIFFERENCE
	cm.	*cm.*	*cm.*
1	98.4	108.0	9.6
2	88.1	93.5	5.4
3	65.4	72.4	7.0
4	101.0	105.0	4.0
5	108.0	115.0	7.0
6	86.0	90.0	4.0
7	96.6	103.0	6.4
Mean			6.2

Since the animals gained in weight and increased in length it may be presumed that they were not seriously injured by the experimental conditions to which they were subjected.

2. General character of the blood. The blood drawn from the left side of the heart is bright red and that from the right side dark, purplish red in color. The general appearance of the blood is quite similar to that of the dog or man. The mean volume percentage of corpuscles is 14.5. Under the microscope the red corpuscle is a pale yellow, oval cell, with a large nucleus. The average sizes of the erythrocytes are given below.

Length	24.89–26.52μ
Width	12.24–16.32μ
Thickness at center	4.08μ

The corpuscles, thus, are larger than human corpuscles. The average number of red corpuscles in the seven animals studied is 850,000 per cu.mm. of blood. The extreme range in different individuals was 616,000 to 1,500,000. The observations from which these averages are obtained are given in table 2.

A leucocyte count was made on alligator 5 in the latter part of March, 1922, and was found to be 8,000 per cu.mm. of blood. The red count at that time was 760,000 per cu.mm. Toison's solution was used in making these blood counts since the leucocytes are stained by the methyl-violet in this solution. A year later a blood count was made on the same animal by Miss Morrison of the Vanderbilt Clinic. The count then was 760,000 for the red blood cells, but 446,000 for the

TABLE 2

Corpuscular volume and red cell count

NUMBER OF ANIMAL	CORPUSCULAR VOLUME		RED CELL COUNT	
	Date, 1922	Volume, per cent	Date, 1922	Number per cu. mm.
1	5–11	17.8	3–28	680,000
2	4–11	13.7	3–21	732,000
2	5–2	13.1	4–11	616,000
4	2–23	16.0		
5	2–23	15.8	3–23	730,000
5	4–6	14.0	3–21(1923)	760,000
6	3–2	11.4	3–13	881,000
6	5–9	11.4	3–30	800,000
6			4–18	745,000
7	4–18	16.1	3–13	1,480,000
7	5–9	15.5	4–4	1,130,000
7			4–25	1,050,000

leucocytes. Leucocyte counts were not made during the year, but a great increase in the number of leucocytes was observed when the red cells were counted. The ratio of leucocytes to red cells, on these determinations, is as 1 to 95, at the first observation, and about 1 to 2 on the second. In man, the values in normal blood are 5,000 to 9,000 per cu.mm. of blood, and about 5,500,000 erythrocytes, or a ratio of 1 to 600 or 1,100. Anything above 10,000 may be regarded as pathological, and in spleno-myelogenous leukemia the leucocyte count may rise to 500,000. It is an interesting fact that the animal that gave this high leucocyte count was in an apparently healthy condition. The study of this remarkable leucocytosis is being continued.

3. Blood gases. The oxygen contents and capacities of the blood and the carbon dioxide contents and capacities were determined throughout the year on six animals.

In following the oxygen content of the blood of an individual animal through a series of observations it is necessary to show that previous hemorrhage has presumably not modified the succeeding determinations.

Whipple (26) has shown that the hemoglobin is replaced in the dog following a hemorrhage of 50 per cent after about three weeks. No work upon the blood volume of the alligator has been reported but

TABLE 3

DATE	NUMBER OF ANIMAL	OXYGEN CAPACITY PER 100 CC. BLOOD		IRON PER 100 CC.
		By gas analysis	From iron	
		cc.	*cc.*	*mgm.*
17–10–22	1	13.2	14.0	34.2
12– 9–22	5	13.0	13.6	34.1
19– 9–22	5	11.2	11.8	29.7
13–10 22	6	12.4	12.2	30.5
3–10–22	7	10.1	10.4	26.1

TABLE 4

Seasonal changes in the gases and in the sugar of the blood

		1922						1923
		Jan.-Feb.	Mar.-April	May-June	July-Aug.	Sept.-Oct.	Nov.-Dec.	March
Volume per cent:								
Oxygen capacity	Mean	13.7	10.6	9.5	9.9	11.8	12.2	15.3
	St. dev.	2.5	2.0	2.5	1.3	1.4	0.6	2.7
Oxygen contents	Mean	11.0	5.5	1.8	3.5	4.3	5.7	10.9
	St. dev.	1.5	1.4	0.2	1.6	1.2	2.7	1.0
Carbon dioxide capacity	Mean	49.9	67.8	53.2	48.3	47.3	45.9	54.3
	St. dev.	5.9	5.3	12.3	3.7	7.1	3.6	7.6
Carbon dioxide contents	Mean	38.6	60.6	48.6	44.4	40.7	42.6	49.4
	St. dev.	5.5	4.9	12.6	5.5	3.8	2.4	16.1
Sugar in mgm. per 100 cc. of blood	Mean	97.0	95.2	92.8	83.0	65.7	29.6	58.0
	St. dev.	27.7	23.6	21.5	26.4	11.8	10.8	10.2

Fry (27) has shown that of a lizard to be 3 per cent and of the frog to be 4.6 per cent of the body weight. If the blood volume of the alligator is assumed to be of this order, we have at no time drawn more than 12 per cent of the total blood volume, and as two weeks were allowed for recovery, it is felt that hemorrhage of itself was not a disturbing factor.

To check the accuracy of the method of gas analysis, the iron of the blood was estimated by the method of Brown (13). The oxygen capacity of the blood was then calculated from the iron and compared with that obtained by gas analysis. These figures are shown in table 3, and a satisfactory agreement is apparent.

4. Seasonal changes of blood gases. The mean values for the gases of the entire series, grouped in bi-monthly periods, are shown in table 4. A survey of the figures for oxygen capacity arranged in bi-monthly periods (table 4), shows that the oxygen capacity reaches a maximum in the early spring, and is lowest during the late spring and summer months. The value found in March of the second year is higher than that of the preceding summer, and is slightly higher (by about 5 volumes per cent) than that of the corresponding period of the preceding year. This increase is very probably due to the stimulation of the formation of hemoglobin due to repeated hemorrhage to which the animals had been subjected, and which is known to occur in birds and mammals (28).

The oxygen contents for the period of January and February have a value of 11.0 volumes per cent; in March and April of the same year of 5.5 per cent; and for May and June of 1.8 per cent, which is the lowest value observed. Thereafter there is a gradual increase until the value of 5.7 volumes per cent is attained in the November and December period. In the following spring the values are again high giving an average of 10.9 per cent for the series.

The oxygen unsaturation of the blood varies from 20 per cent during the January and February period through 58 per cent for March and April, to 81 per cent in May and June. From this it progressively decreased to 46 per cent for November and December. Determinations were not obtained during January and February of the second year but those made early in March gave a value of 29 per cent.

Any interpretation of these results must take into account the fact that the animals were kept at the approximate temperature of 26.5°C. No observations of the blood gases of the alligator in its natural habitat have been made but the differences we find during the year are such as we might expect to find if the animals were subjected to seasonal changes. In cold weather, the metabolism and consequent use of oxygen are at a low level and the oxygen unsaturation of venous blood would be slight. In summer months when metabolism is more rapid, we would expect the oxygen unsaturation also to be correspondingly greater, since the oxygen is consumed to a greater extent.

We must interpret these results as due to a periodicity acquired by the animal in its former habitat, or perhaps to some natural, internal rhythm of the animal, but certainly not to the immediate environment in which the animals lived during the period of observation.

The blood of different animals when exposed to carbon dioxide at a tension of 40 mm. of mercury shows a varying capacity for fixing this gas. The carbon dioxide capacity is distinctly higher in the early spring than during the remainder of the year. This is illustrated in table 4, from which it appears that the carbon dioxide of the March and April period is well above that of the other periods. The fact that the volume for the second March is lower than the corresponding earlier period is possibly due to a partial loss of the periodicity resulting from the prolonged period in which the animals were subjected to constant temperature conditions.

The carbon dioxide contents show a similar behavior. In the March and April period, carbon dioxide content is greater than in the winter or summer months. The lowest period seems to be in the fall and winter. In the second spring the value is higher than during the preceding winter and summer, but is not as high during the first spring. Animals 4,6 and 7 gave high values while the other three animals were low. The fact that some of the animals do not show an increase at this time of the year, while some do, is possibly due to a gradual loss of the periodicity due to constant conditions.

5. Gaseous nitrogen of the blood. The gaseous nitrogen of the blood was found to be higher than in any animal so far reported. In human blood, the average value for the nitrogen is 1.36 volumes per cent (29). In the horse, Regnard and Schlosing (30) found the nitrogen to be 2.00 volumes per cent. On ten determinations the mean value for the alligator was 0.033 with a standard deviation of 0.0055, on 1 cc. of blood, giving a value of 3.3 volumes per cent.

6. Chlorides. The chloride content of the blood of the alligator was found to be 525 mgm. per 100 cc. of blood. The value for dog's blood is about 550 to 570 mgm. per 100 cc.; for ox blood about 450 mgm., and for human blood 650 mgm. per 100 cc. The urine appeared to be wholly free from chlorides, not a trace being found at any time.

7. Relative efficiencies of erythrocytes of alligator and man. It was of interest to compare the relative efficiency of the erythrocytes of the alligator and man as oxygen carriers. This was done by comparing the actual size of the red corpuscles of the alligator with those of man, and at the same time, comparing each with respect to oxygen carrying power. From this it may be possible to form some idea of the physiological efficiencies of the cells of the two types of animal.

The volume of each erythrocyte may be calculated by dividing the corpuscular volume by the number of red cells. Thus in man, the corpuscular volume may be taken as 40 per cent (31) and the number of red cells per cubic millimeter as 5,500,000 (31). The volume occupied by one corpuscle would be 0.4 cu.mm. divided by 5,500,000 or 7.2 $\times$

10^{-8} (32). In the alligator a wide range in the number of red cells was found, but the average number per cu.mm. is 850,000, and the average corpuscular volume per cent is 14.5. The volume of the individual corpuscle, therefore, is 0.145 cu.mm. divided by 850,000 or 16×10^{-8}. The alligator corpuscles therefore compare in volume with the human corpuscles as $\frac{16 \times 10^{-8}}{7.2 \times 10^{-8}}$ or as 2.2: 1.

The oxygen capacity of human blood is about 19 cc. per 100 cc. of blood. The factor for converting oxygen capacity into hemoglobin is 0.746. The hemoglobin content of human blood is 14 to 15 grams per 100 grams of blood. The oxygen capacity of alligator blood may be taken as 12 cc. per 100, and the hemoglobin content would then be about 9 grams per 100 grams of blood. If a proportional amount of hemoglobin were carried by the two kinds of erythrocytes, the corpuscular volume would also be proportional. That is 9: 14.5 as X: 40. The corpuscular volume of the alligator under these circumstances would be about 25 volumes per cent. The corpuscular volume was found, however, to be 14.5 per cent and thus 25 14.5 represents the ratio between the hemoglobin carrying powers of the two kinds of corpuscles. The alligator corpuscles then either contain relatively more hemoglobin than human corpuscles, or in some way the hemoglobin is more efficient. From this we may conclude that one alligator corpuscle is equal in volume to 2.2 human corpuscles and in its efficiency as an oxygen carrier is 1.7 times that of the human corpuscle. So it would take about four human erythrocytes to equal one alligator corpuscle as an oxygen carrier.

8. Comparison of the gases of arterial and venous blood. In order to compare the gaseous content of arterial and venous blood, samples were drawn successively in the manner described above, with the minimum possible time interval between samples, since it was obviously impractical to draw the two samples simultaneously. This was done, perhaps unfortunately, at the season of the year when the animals showed the maximum variation between individuals. However the results are interesting and are included as the first observations of the kind upon the alligator.

The detailed results are shown in table 5. The carbon dioxide contents, given by alligator 4, which alone gave the average value for this season as shown in table 4, for venous blood was 38.4 volumes per cent, and 32.0 volumes per cent for arterial blood, a difference of 6.4. Similarly, the oxygen content of venous blood was 11 volumes per cent and for arterial blood 15.6 volumes per cent, and the difference was 4.6.

Similar data are not available for many species though Buckmaster and Gardner (33) publish some figures for the cat, and Hastings (34),

among others, for the dog, while there are, of course, many observations upon man (35). For purposes of comparison some of these data are included in table 6. It must be borne in mind that values of this kind are never absolute but must vary with the nature of the metabolism of the several individuals at the time that the samples were taken.

Peters, Barr and Rule (36) quote respiratory quotients in human blood, of 1.62 to 0.34, and Harrop (37) reports values of 1.81 to 0.62.

9. Carbon dioxide absorption curve. Oxalated blood was exposed to atmospheres containing known percentages of carbon dioxide and air. Samples were removed and analyzed after equilibrating the blood with

TABLE 5

Arterial and venous blood of the alligator

NUMBER OF ANIMAL	DATE	VENOUS		ARTERIAL		DIFFERENCE		R.Q.
		CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2/O_2
3	2–16–23	33.0	10.5	25.8	13.7	7.2	3.2	2.2
4	2–16–23	38.4	11.0	32.0	15.6	6.4	4.6	1.4
6	2–13–23	66.5	10.0	59.1	14.6	6.5	4.6	1.43
7	2–15–23	48.3	4.1	41.1	10.1	7.2	6.0	1.2
Mean						6.8	4.6	1.47

TABLE 6

Gases in volumes per cent in arterial and venous blood of several animals

	VENOUS		ARTERIAL		DIFFERENCE		R.Q.
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2/O_2
Human	48.0	12.0	40.0	20.0	8.0	8.0	1.00
Dog	40.5	14.3	38.3	19.3	2.2	6.0	0.37
Cat (etherized)	40.8	9.9	25.07	13.6	15.77	3.7	4.2
Alligator 4	38.4	11.0	32.0	15.6	6.4	4.6	1.4

the gaseous mixtures. The results are plotted in figure 2, by placing tensions (calculated from percentages) of carbon dioxide in millimeters of mercury, on the abscissae and volumes per cent of carbon dioxide absorbed by the blood, on the ordinates. Determinations of this kind were made on the blood of each of three animals. The three curves fall at different heights as would be expected from the differences in alkali reserve already noted. They are, however, approximately parallel and the middle curve does not depart greatly from that for human blood published by Christiansen, Douglas and Haldane (38), which is drawn from the mean of many observations and which is represented by plus marks (+) in the figure. From this one might conclude that the mechanism of carriage of carbon dioxide in the blood of the alligator is essentially the same as that of human and mammalian blood, in general.

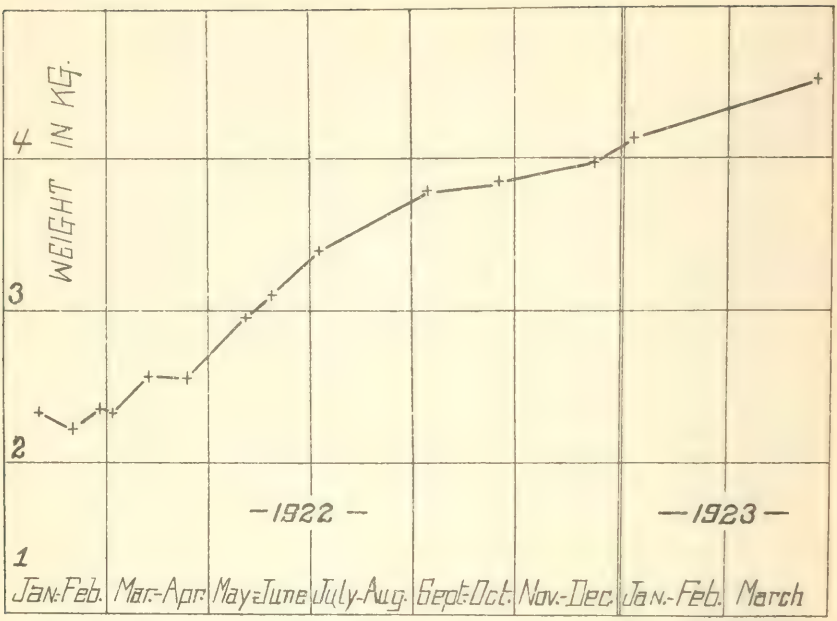

Fig. 1. Weight curve for alligator 1. The curves for the other individuals were essentially parallel to this. Only in two cases did the curves of the different individuals cross.

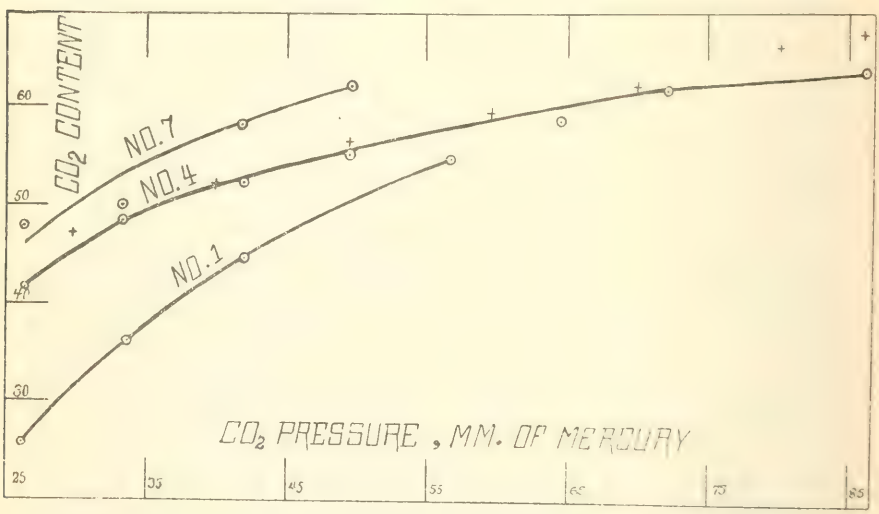

Fig. 2. Carbon dioxide dissociation curve for the blood of three alligators (circles) compared with that for human blood (crosses).

It might be further noted that the variations in alkali reserve among the individuals at this season are no greater than those found by Hastings (34) for different dogs.

10. Seasonal changes in the concentration of sugar in the blood. The concentration of the sugar in the blood of these animals was determined throughout the year. The mean values arranged in bi-monthly periods are shown in table 4. Animal 3, though subjected to the same general conditions, was used very little in the routine work of the study and served in the nature of a control on the disturbances due to the experimental procedure. The blood sugar in this animal was 97 mgm. per 100 cc. of blood in the January–February period, 92 mgm. in the July–August period, and 59 mgm. in the November–December period. In March of the second year the value was 92 mgm., about the same level as at the beginning of the experiment. The reaction of this animal was of the same nature as that of the other animals, but more marked, as will be seen upon comparison of the figures of this animal with those given in table 4.

A high sugar content is shown by all of the animals during the late winter and early spring. During the late spring a distinct drop in the sugar is noted, which continues during the summer and autumn, the lowest values being obtained for the November and December period. At this time, the mean for all the animals was only 37.5 mgm. per 100 cc. of blood.

Since the earlier determinations gave an average of about the same magnitude as is common in the mammals, i.e., 97 mgm., it is of interest to compare the low value with the "critical value" of 44 mgm. described by Banting, Best, Collip, Macleod and Noble (39). It should be noted that during this "low" period the alligators appeared in every way normal and so far as one could determine by appearance, were in the same physiological conditions as when the blood sugar was at its highest. The work reported by Riddle and Honeywell (40) for the pigeons indicates that the blood sugar of at least some forms can be reduced to a very low level indeed (25 to 30 mgm.) without evoking the disturbances which have been described for those of the mammals which have been studied. Alligator 1, on November 21, 1922, showed no sugar in its blood, though at the same time it appeared otherwise to be in normal condition, and on March 7, 1923, yielded a value of 113 mgm. This reminds one of the behavior of the dogfish where the sugar was at times low or absent. (See Scott (41) and Macleod (42).)

The annual cycle in the above discussed data is of much interest from the standpoint of seasonal changes in physiological phenomena, but any interpretation can only be conjectural at the present time.

The work of Riddle and Honeywell (43) shows a periodicity in the accumulation of sugar in the blood of the pigeon accompanying the periodicity of ovulation. The period of ovulation in the alligator occurs only in June, and only animals of mature age (five to ten years) carry out this function (44). All of our animals showed this seasonal rhythm, even the smallest which was hardly two years old, so in this case the periodicity in the blood sugar is evidently not related to ovulation.

The periodicity may be related to the storage of glycogen shown to occur during autumn and winter in the marmot by Claude Bernard (45) and in the frog by Moseik (46), since we found the sugar to be low at the season of the year that the glycogen in these animals is high.

In plants an accumulation of reserve substances occurs during the fall and winter, and a mobilization of these in a diffusible form occurs in the late winter and early spring (47), and in this respect may resemble a poikilothermic animal that passes through a winter period of lowered metabolism.

In all cases where the behavior of these alligators is related to hibernation, it is necessary to remember that they must have carried their periodicity over from their former habitat which was northern Florida, and hence our late winter period would correspond to their early spring.

In addition to any such conjectural considerations it is of course necessary to add the possible effects of the temperature changes in concentration of the blood sugar which may be presumed to be the results of such more immediate physiological equilibration.

11. Discussion of seasonal changes. There has grown up a very considerable literature on seasonal variations of physiological functions in various animals. In this literature it is developed that there are very few if any organs or physiological systems that are not affected by this factor in one or another of the animal groups. This is not the time for an extensive reivew of the literature though a brief review of some of the papers on variations in the respiratory system, the metabolism of the carbohydrates and the persistence of the seasonal variations after the animals have been removed from their natural and variable environment to the more or less perfectly controlled conditions of the laboratory may not be out of place. From the foregoing discussion it will be remembered that there was a periodicity in the concentration of the gases and the sugar of the blood of the alligator, which must be related to previous effect of changes in the seasons of the year, and not to the immediate environment of the animal.

The respiratory exchange in the lungs of the frog under laboratory conditions was found by Athanasiu (59) to varying according to the season of the year. He found that the respiratory quotient was greater in the winter months than in the summer.

During hibernation of the woodchuck, Rasmussen (48) found a gradual increase in the amount of carbon dioxide in venous blood from about 70 volumes per cent to as high as 100 volumes per cent. His results supported those of Dubois (49). When the animal wakes from its winter sleep the carbon dioxide falls very rapidly to its lower level.

In the frog there is a seasonal change in the storage of glycogen in the liver and other organs of the body. Mozeik (46) has shown that in summer there is scarcely a trace of glycogen in the liver, but in the late autumn the liver is extremely rich in glycogen. This observation was confirmed by Athanasiu (50). The period of high glycogen content in the frog corresponds to the period of low sugar content of the blood in the alligator.

TABLE 7

Nitrogen distribution in the urine

NUMBER OF ANIMAL	DATE	SP. GR.	MGM. PER 100 CC.				PER CENT TOTAL N		
			N	NH_3	Urea-N	Uric acid-N	NH_3	Urea	Uric acid
2	7–27	1.005	219	171	7.0	39.0	79.5	3.3	17.0
2	7–26	1.0062	249	198	3.3	28.0	80.0	1.3	11.0
1	8–4	1.0063	225	148	13.0	24.2	66.0	5.8	10.8
5	8–7	1.0048	268	208	16.0	18.4	77.0	6.0	7.0
After sterile catheterization									
1	8–14	1.0049	208	168	0	41.3	81.0	0	19.8
1	11–8		207	140	20.5		67.0	10.0	
1	11–9	1.0053	202	154	35.0		77.0	17.0	

Claude Bernard (45) showed that in hibernating animals there is an increase in glycogen up to and including the period of winter sleep. The glycogen rapidly disappears when the animal awakes.

Physiological processes may be modified by a diurnal or annual cycle independently of the experimental conditions.

12. Distribution of the non-protein nitrogen in the blood and urine. The urine was collected by direct catheterization of the cloaca. The evidence that the urine so obtained was not contaminated by the feces and was in fact pure urine consists of the fact that it was always found to be a clear, limpid fluid, and that the specific gravity was found to be as consistent as would be expected. See table 7.

Analysis of the nitrogenous constituents of the urine always showed a high percentage of ammonia, in all cases more than 50 per cent of the total nitrogen appeared in this form, and occasionally more than 85 per cent.

Urea was usually present, in amounts varying up to 17 per cent of the total nitrogen, but was occasionally absent. Extra precautions for sterile catheterization tended to increase the proportion appearing in this form, but not in significant amounts.

Seven to 20 per cent of the total nitrogen of the urine appears as uric acid. The low percentage of uric acid is of interest in view of the common statement that the nitrogen metabolism of the reptiles is similar to that of the birds.

Wollaston in 1810 (51) showed that uric acid occurs in large amounts in the excrement of birds. Since this time, it has been shown by the investigation of a large number of workers that uric acid in birds arises from *a*, lactic acid and ammonia; *b*, from amino acids; *c*, from the purine bases, and *d*, from urea. Hoppe-Seyler showed that the excrement of certain lizards may consist almost wholly of uric acid (52), and Salkowski (53) mentioned the excrement of most snakes as a convenient source of

TABLE 8

Uric acid in blood and urine

NUMBER OF ANIMAL	BLOOD		URINE		
	Date	Mgm. uric acid per 100 cc.	Number of animal	Date	Mgm. uric acid per 100 cc.
1	4-20	1.67	1	7–25	36.8
1	5–11	2.25	1	7–26	37.6
4	5–4	2.56	1	8–2	27.8
5	4–27	1.83	1	8–3	23.6
5	5–18	2.60	1	8–4	24.2
6	5–9	1.79	1	8–14	41.3
7	5–16	2.24	1	10–24	22.8

obtaining uric acid in the pure form. The excrement of certain Pythons was shown to consist of ammonium urate (54). Snakes and lizards appear, therefore, to be similar to the birds in regard to the main end product of their protein metabolism. That this is not true of all forms of reptiles is shown, however, by the work of Karr and Lewis (55), who found large quantities of urea in the blood of the turtle. It is not true of the lower vertebrates, for nitrogen is largely eliminated by frogs (56) and fish (57) as urea. The facts just cited have given rise to the generalization that animals living in dry habitats, birds, snakes, lizards, excrete nitrogen as uric acid, while those living in water, fish, frogs and turtles, excrete it as urea instead. To this latter list we had hoped to add the alligator, but all we can say at the present time is, that it does not excrete large amounts of uric acid, and may possibly take an intermediate position between the lizards and the turtles.

The urine was neutral or faintly alkaline on drawing, but rapidly became strongly alkaline on standing, and a crystalline deposit of calcium carbonate and triple phosphates was thrown down. When collected under oil the urine showed a very high content of carbon dioxide, as much as 250 cc. per 100 cc. of urine. The change of reaction on standing is probably due in part to loss of CO_2.

We have found nothing in the literature on the nitrogen of the blood of the alligator, although Krukenberg (58) found creatine and hypoxanthin in the muscle extract of the alligator. We had hoped that analysis of the nitrogen constituents of the blood would throw light on the origin of the large quantities of ammonia in the urine. Contrary to our expectation, no urea was found in the blood. That this was not due to faulty technic is indicated by the fact that known amounts of urea added to the blood were recovered, and that the accepted values were found for beef blood. Less than 15 per cent of the non-protein nitrogen of the blood is in the form of ammonia. In other cases, where uric acid

TABLE 9

Nitrogen distribution in the blood

NUMBER OF ANIMAL	DATE	MGM. PER 100 CC.			PER CENT TOTAL N
		Non-protein N	NH_3	Urea	NH_3
2	1–4	15.0	2.1	0.7	14.0
1	1–5	15.0	2.1	0	14.0
7	1–9	19.6	2.1	0	10.7
4	1–10	18.0	2.1	0	11.7
6	1–11	16.0	2.1	0	13.0

alone was determined, the quantity of uric acid was found to average 2.13 mgm. per 100 cc. of blood, which represents only about 4 per cent of the non-protein nitrogen.

Neither creatine nor creatinine were found in either blood or urine.

The total nitrogen of the blood was found to be about 2000 mgm. per 100 cc. of blood.

The apparent absence of urea and the relatively small quantity of ammonia in the blood may indicate that there is some unknown precursor of the urea and ammonia which is found in the urine. The small amount of uric acid present in the blood would indicate that this precursor is probably not uric acid.

I wish to express my great appreciation of the continual assistance given me by Professor E. L. Scott of this Department, in carrying out this investigation.

SUMMARY

1. The erythrocytes of the alligator average 850,000 per cu.mm. of blood. They are about 14 μ in width and about 25 μ long, and are oval, nucleated cells.

2. The average corpuscular volume is about 14 to 15 per cent.

3. The oxygen capacity of the blood varies according to the season of the year between the values 9.5 and 13.7 volumes per cent. The oxygen content varies from 1.8 to 11 volumes per cent. The oxygen unsaturation of the venous blood is slight in late winter (20 per cent) and is very great in the summer (80 per cent).

4. The iron content of the blood is about 30 mgm. per 100 cc.

5. The carbon dioxide capacity and content of blood also show a seasonal variation, being about 20 volumes per cent higher in early spring than during the summer and winter.

6. The carbon dioxide absorption curve of alligator blood is essentially the same as that of human blood.

7. The sugar content of the blood shows a seasonal variation, being high in the spring and very low during late fall and winter.

8. Uric acid is present in only small quantities in the blood and urine of the alligator.

9. The main nitrogen constituent of the urine is ammonia. The precursor in the blood of the ammonia in the urine is not known.

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THE BEHAVIOR OF BLOOD-SUGAR VALUES IN HEREDITY

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The behavior in crosses of a character similar to or comparable with the normal sugar level of the blood has hitherto not been investigated. Earlier physiological work indicates that this is a character which is normally maintained at a given level throughout the whole of the life cycle so far as this has been studied; probably it is normally essentially constant for the species or race; in the individual it is much modified in certain diseases, and it shows very wide temporary fluctuations under a variety of external and internal conditions; finally, changes in blood sugar concentration are necessarily accompanied by other changes of concentration or of equilibrium not only in the blood itself but in the living tissues which must in turn equilibrate or adjust to changes in this circulating tissue. If, therefore, from a cross of different species or genera, a *hybrid* with a definitely new or different sugar level could be formed, all this hybrid's characteristics would be forced to undergo practically the whole of their development in an intimately surrounding medium different from that of its parents. Possibly we should thus have an opportunity to observe the response of the parental characters to this change (and doubtless of course to some or many other unknown or unstudied simultaneous changes) in the blood.

Studies on the inheritance of visible characters in crosses of widely separated forms—of distinct species, genera or families—have shown in some cases (Whitman, 15) marked irregularities and behavior of characters elsewhere known to segregate and show complete dominance or complete recessiveness. In other cases simi-

larly wide crosses of other biological types have apparently failed to show notable departures from the expected behavior of these same characters. To us it seems probable that the results of the present study throw some light on the reason for the aberrant behavior of these visible characters in some generic crosses and also for the normal behavior of these same characters in other generic crosses. Whether justified in this speculation or not we are now able to establish one or two facts concerning the hereditary behavior of blood sugar values in two pairs of generic crosses.

The possibility of studying the behavior of blood sugar values in inheritance has only recently become apparent. This possibility has arisen in part through the fuller recognition and partial elimination of several factors which cause wide temporary fluctuations in the sugar values obtained from pigeons (6, 9, 10, 11, 12); and more important still from our demonstration (6, 9, 10) that different genera and families of pigeons—previously known to be fertile in crosses—have distinctly different normal levels of sugar in their blood. A fortunate aspect of those results presents itself in the circumstance that the two species of the two genera which previously had been most extensively used in genetic and sex studies by one of us were there shown to have almost the highest (*T. orientalis*; see Table II) and quite the lowest (*St. alba*) blood sugar values obtained from the several genera examined. And from this cross a sufficient number of F_1 hybrids and of F_1 back-crossed with one parent species were at hand for a partial investigation of this problem. From still another generic cross the two parents and 37 of their offspring supplied material for a second test.

Materials and Methods

After other studies had made it clear that, of the several species of pigeons studied by us, the Japanese turtle dove (*Turtur orientalis*) has almost the highest concen-

tration of blood sugar and the ring doves (*Streptopelia alba* and *St. risoria*) have the lowest, it was decided to use all the adult hybrids of this cross then in our collection as the chief material of the present study. These hybrids were aged 17 to 183 months. From the 81 F_1 hybrids taken for study we obtained duplicate sugar determinations on 38 individuals which proved to be free of observable disease at autopsy. All the birds used in the study were killed after drawing the last blood sample to discover possible disease and to make sure that no ovulations were imminent in the females (6, 9). For hybrids other than the F_1 the available number of healthy individuals was considerably less—only 7 were "five eighths" Turtur hybrids; 8 were "one fourth" Turtur; 8 were either "one thirty second" or "one sixty fourth" Turtur and these are combined here and treated as one group. A study of a group of "one eighth" Turtur hybrids was delayed until after the onset of cold weather (October 19). Before this work could be carried out other studies had shown (11) that the blood sugar is considerably affected by the onset of cold weather in the autumn and the proposed study of this group of *Turtur* hybrids was therefore abandoned. The 10 *Turtur orientalis* and the 10 *Streptopelia* listed in Table I as "parent species" (all aged 10 to 47 months) are in most cases not the actual parents of any hybrids represented in the table; these hybrids arose from numerous matings of individuals of these genera, and in most cases the parents of the hybrids were not alive at the time this study was begun. The sugar determinations for the *Turtur* and *Streptopelia* "parents" and for the several groups of their hybrids were all made between April 28 and October 15. Except for a very few individuals this period ended on August 17.

A second group of F_1 generic hybrids—a large single fraternity from a cross of a *Spilopelia* male with a *Streptopelia* female—was also studied; and in this case the sugar values of the actual parents and of the frater-

nity of the female parent were determined. Autopsy showed, however, that only 20 of the 37 F_1 individuals from this generic cross were healthy; there being 7 tuberculous birds and 10 others infested with round worms. Several of these diseased birds gave very aberrant sugar values. The male *Spilopelia* parent was an imported (tropical) bird of uncertain age but certainly more than 3 years old and fully fertile in this generic cross. The female parent was 25 months old. Their hybrid offspring were all hatched in the same year and were aged 6 to 16 months when taken for this study. All the sugar determinations for all members of this group of birds were made between June 17 and August 17.

All the blood samples (except 12 *Streptopelia* and 12 *Turtur*, included in Figure 2, from samples obtained from the upper beak) were drawn by needle-puncture of the heart, and duplicate samples were taken usually at intervals of one day. The heart-punctures sometimes resulted in the death of the bird at the drawing of the first sample. This accounts for the "single determinations" separately listed for the F_1 hybrids in Table I. An examination of the distribution of deaths from nearly 1,000 heart-punctures indicates that death more frequently resulted in (a) diseased birds, and (b) in birds carrying eggs nearly ready to lay or to ovulate. The prevalence of disease in these birds and the questionable value attaching to an unchecked determination warrants our exclusion (or separate presentation) of the values thus obtained.

Since our earlier work (6, 9) has shown that the blood sugar increases in the female at the ovulation period, and since the heart-puncture was found occasionally to cause the resorption of an ovum in the ovary or to cause it to pass into the body cavity, it was necessary to obtain samples in females with resting ovaries and also necessary to autopsy the birds within one to three days of sampling in order to make sure that the ovaries were really resting. And since diseased birds should ob-

viously be excluded, or be given separate consideration as is done here, it was necessary to kill the males also. It is of course only disease discoverable to us at autopsy that has been excluded, and probably a very few birds otherwise diseased are included in our groups of apparently healthy birds. Only non-ovulating sugar values enter into the data presented here. The sugar determinations were made by the Maclean micro-method. Further details of method, including precautions taken in the handling of the birds, have been described elsewhere (5, 9).

DATA

Since the actual amount of sugar in the blood is subject to some fluctuation, notwithstanding the several precautions taken by us, and because of the small but appreciable error in the method of determination itself, and finally because any individual may have been significantly even though not discoverably diseased, it is usually necessary in a study of the present kind to attach more significance to results obtained for a *group* than to even well-checked results for an individual. This is a most serious limitation if the end in view were a complete and definite account of the behavior of a character in inheritance. But a thorough analysis lies wholly outside the scope of our purpose, since besides various limitations of method and material no F_2 generation can be reared from these generic hybrids, and we must deal chiefly with the P_1 and F_1 generations. We do, however, attempt a small amount of individual treatment of the data obtained from the two F_1 series studied by us.

Table I gives the details of results obtained from the various groups concerned in the cross of *Turtur* with *Streptopelia*. It will be noted that for 10 pure *Turtur orientalis* which proved healthy at autopsy we find 188 mgms sugar per 100 cc of blood. That this very high sugar value is not in excess of what we may accept as normal for this species is further indicated by the fact

TABLE I

SUMMARY OF BLOOD SUGAR DETERMINATIONS ON TWO PARENT SPECIES BELONGING TO DIFFERENT GENERA; ON THEIR MALE AND FEMALE F_1 RECIPROCAL HYBRIDS; AND ON HYBRIDS BACK-CROSSED TO ONE PARENT SPECIES

	Description of birds	Sex	No. of birds in group	Average (or mean) Body weight	Average (or mean) Milligrams sugar per 100 cc. blood
Parent species	Turtur orientalis	♂ + ♀	10	221	188
	Streptopelia alba-risoria	♂ + ♀	10	171	149
One half T. orientalis (F_1)	All duplicate sugar determinations				
	Hybr. (♂ Turtur × ♀ Strep.)	♂	17	199	174
		♀	13	194	169
	Hybr. (♂ Strep. × ♀ Turtur)	♂	16	203	166
		♀	19	184	158
	Mean of 4 groups				*167*
	All single sugar determinations				
	Hybr. (♂ Turtur × ♀ Strep.)	♂	4	191	150
		♀	2	188	155
	Hybr. (♂ Strep. × ♀ Turtur)	♂	4	202	155
		♀	6	187	163
	Mean of 4 groups			*192*	*156*
	Healthy birds only and duplicate sugar determinations				
	Hybr. (♂ Turtur × ♀ Strep.)	♂	12	194	174
		♀	9	197	169
	Hybr. (♂ Strep. × ♀ Turtur)	♂	9	204	165
		♀	8	191	160
	Mean of 4 groups			*196*	*167*
Five eighths Turtur orientalis[3] (♀ 1/8 Turtur back-crossed with ♂ Turtur)		♂	1	199	16[illegible]
		♀	6	203	174
One fourth Turtur orientalis[3] (F_1 back-crossed to ♀ Strep.)		♂	4	170	164
		♀	4	166	151
	Mean of 2 groups			1[illegible]	*158*
One thirty second or one sixty fourth Turtur[3] (back crosses to ♂ and ♀ Strep.)		♂	2	171	164
		♀	6	155	151
	Mean[4] of 2 groups			1[illegible]	*157*

[3] Healthy birds only.

[4] The "mean" signifies the unweighted average of the two (or four) average values.

that 12 additional birds thought to be healthy, but too valuable to kill (used in Figure 2), gave a value of 190. And the seven additional diseased birds examined gave an average of 198 mgms; two of this group gave extremely low and four gave very high values. It has been shown by still other work that the ring doves (*Streptopelia*) are properly measured by the 149 mgms obtained from the 10 healthy individuals listed in Table I. This value is sufficiently confirmed by figures obtained for the two following groups: First, the 12 apparently healthy birds (too valuable to kill) added to the 10 known healthy birds in Figure 2; these gave an average of 150.6. Second, three small fraternities (parent and grand-parents) shown in Figure 3 gave a like result; only *Streptopelia* is represented there. The fraternity of the male grand-parent (*St. risoria*) gave a value of 152, the female grand-parent (*St. alba-risoria-douraca*), 148, and the parent fraternity, 147. These 14 *Streptopelia* again average 149 mgms sugar per 100 cc of blood. In a cross of these species of *Turtur* and *Streptopelia* it is evident that we are making a cross of sugar values which rather closely correspond to 188 and 149 respectively.

Three readily understood divisions of the data obtained for the F_1 hybrids are given in Table I. In each division four groups represent the necessary separations for the sexes and for reciprocal crosses. The third or last of these principal divisions should supply the most reliable results. It will be noted, however, that the larger numbers of (duplicate) determinations of the first division gives a result practically identical with that shown in the third division, although the first division includes a number of diseased birds. But the second group—including smaller numbers, unchecked determinations and a high proportion of diseased birds—gives values for both blood sugar and for body size which do not check well with the more complete and more reliable data. It is, nevertheless, notable that all of the four groups of this defective division, as well as of the eight groups of

the two other divisions, *show a sugar value for the hybrids which is intermediate to those of the two parent species which entered the cross.* The mean value (167 mgms) obtained for the third (and first) division is an almost exact intermediate of the values found for the two parent species—being 21 points less than *Turtur* and 18 points greater than *Streptopelia.*

In a series of "five eighths" Turtur hybrids seven birds gave an average[1] value of 172 mgms. Thus, this group, which stands nearer pure *Turtur* in composition

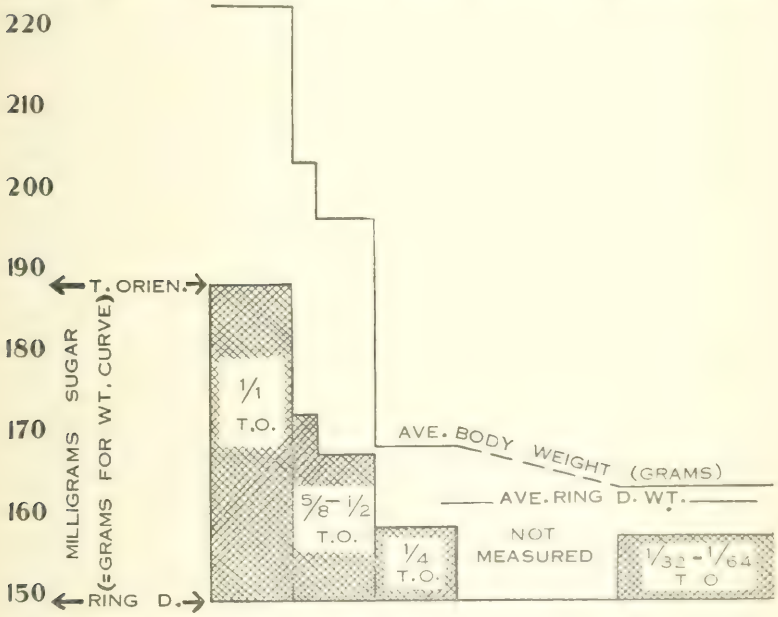

FIGURE 1

The rectangular blocks indicate the amounts by which the blood sugar values of *T. orientalis* and its various hybrids (five eighths; one half; one fourth; one thirty second) from crosses with the ring dove (*Streptopelia*) *exceed* the sugar value of the ring dove parent (the base line).

The uppermost line records the mean body-weight of the identical birds of the group whose mean blood sugar value is expressed in the rectangle below. The very similar and repeated "fractionations" of the body-weight and the blood sugar in the hybrids suggest that the same method of inheritance probably holds for both weight and sugar values.

[1] The *average* is probably better used here than the mean, particularly since there is only one male, and since here only is the male indicated to have a lower sugar value than the female. In the case of all other hybrid groups the *mean* was used in the construction of Figure 1.

than do the F_1 hybrids, also more nearly approaches the sugar level of *Turtur* than do the latter hybrids.

The eight "one fourth" Turtur hybrids—arising from three different matings of F_1 hybrids (F_1 reciprocals both represented) back-crossed with *Streptopelia* females—again give a value (158 mgms) intermediate to that of their parents (F_1 and *Streptopelia*). The last class of these *Turtur* × *Streptopelia* hybrids—including eight birds which were only "one thirty second" or "one sixty fourth" Turtur—gave a mean sugar value of 157 mgms (average, 154) which is again an intermediate between the "one fourth" Turtur hybrid and the pure *Streptopelia.*

The above results are diagrammatically represented in Figure 1. The base line there represents the blood sugar level of the *Streptopelia* parents; and the shaded areas indicate the amount which this is surpassed, first by pure *Turtur* and later by the various hybrids of *Turtur* and *Streptopelia* in the order of their fractional or compositional relationship to *Turtur*. This diagram clearly indicates that the blood sugar value of the hybrids was "fractionated" or divided in the three (or four) graduated dilutions, or divisions, of the amount of *Turtur* represented in the offspring. If the reader wishes to visualize the total absolute sugar values of all groups, instead of their differences, he can do so by imagining the base line (149) carried downward at all points to zero.

A curve for the "body weight" of the several groups of birds which supply the blood sugar data is added to the uppermost section of the figure. This has been done to facilitate a comparison of the behavior of size and blood sugar in inheritance. The marginal figures which indicate "milligrams of blood sugar per 100 *cc* of blood" may be converted into "grams body weight" for the reading of this curve. In order to indicate the base of this curve, *i.e.*, the normal ring dove weight, we have not used the figure given in Table I for the 10 ring doves used there, but the value (161 grams) indicated by a much

larger previous series covering the season at which the majority of the hybrids of this study were weighed and killed.

Since one would like to know something of the individual sugar values and the range of variation which gave the mean intermediate value (167 mgms) for the F_1 hybrids of this cross, we have supplied these data in the

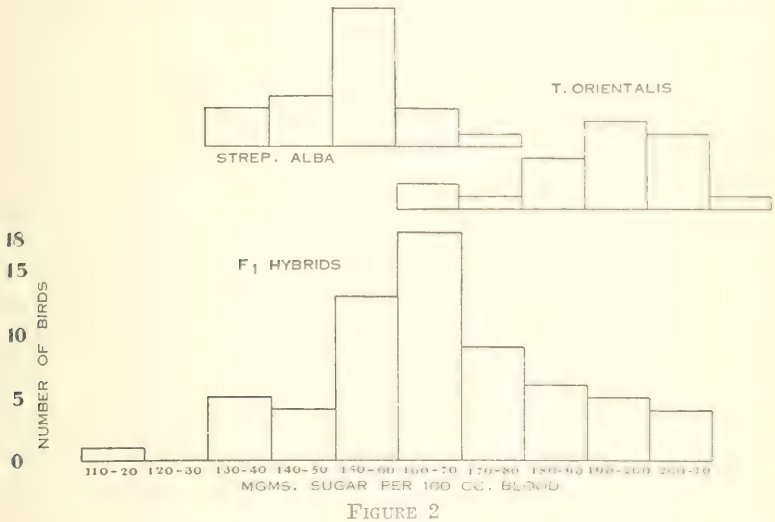

FIGURE 2

Polygons showing distribution of blood sugar values obtained from 65 F_1 *Turtur* × *Streptopelia* hybrids, and from 22 individuals of each of the two parent species (*Turtur orientalis* and *Streptopelia alba*).

form of a polygon in Figure 2. This figure makes it clear that the mode of this polygon corresponds with the mean value (167 mgms; average, 166) and that our mean and average values are not derived from the combination of numbers obtained from a bimodal curve. In other words, the values obtained are distributed as are the values from characters which in F_1 show "intermediate" behavior in heredity. In order to have larger numbers of F_1 for this polygon, all the birds—including some unhealthy ones of the first division of Table I are here utilized. And in order to have larger numbers for the construction of similar polygons for the two parent species we have here added to the 10 healthy individuals listed in Table I, an-

other 12 individuals of each of these species which were all apparently healthy but too valuable to kill and verify this point. The polygon of each parent species therefore includes 22 individuals. The average blood sugar of the 22 *Streptopelia* represented is 151; of the 22 *Turtur*, 190. It is then also clear that these polygons for the parent species show a distribution about a single mode, and that this modal point includes the average blood sugar value found in the group as a whole.

The results obtained from a second generic cross—a male *Spilopelia suratensis* mated to a female *Streptopelia* hybrid—are diagrammatically represented in Figure 3. The blood sugar level of the *Spilopelia suratensis* is known only from the data obtained for the parent (♂ P870) used in this cross; but the value (170 mgms) obtained for this individual is the result of three well-checked determinations. One individual of another species of this genus—*Spilopelia chinensis*—gave 173 mgms sugar. The sugar value obtained for the female parent of the hybrids of the cross now being discussed requires special consideration.

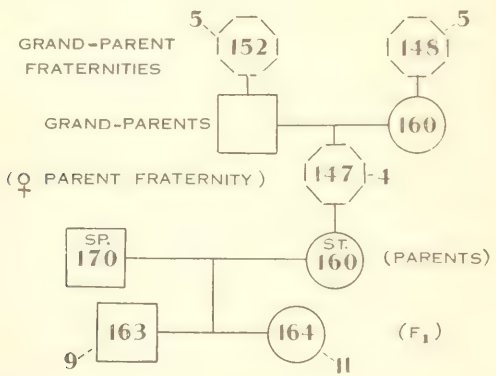

FIGURE 3

Family history, in terms of blood sugar values, of 20 (9 ♂ and 11 ♀) healthy generic F_1 hybrids and of their male (SP. 170) and female parents (ST. 160). These hybrids show an average and a mean sugar value intermediate to the values of their parents and of the genera to which their parents belong.

The blood sugar value given in Figure 3 for the female

parent (P995) is 160 mgms. This is of course the value obtained; but the autopsy revealed that this was not a true non-ovulation value—an unsuspected ovulation into the body cavity having occurred about two days before the blood samples were drawn. Other work has shown (6, 9) that the sugar value increases at ovulation and that about 108 hours must then elapse before the blood sugar again returns to normal. Under these conditions the sugar value obtained was almost certainly too high. But we have the following evidence concerning the sugar value which she would be expected to transmit to her offspring: Sugar determinations made on four individuals of her own fraternity gave an average of 147 mgms; her mother gave a value of 160, but this also was probably abnormally high since five determinations on individuals of her own family (fraternity and parent) gave a value of 148 mgms; again, five members of her father's family gave a value of 152 mgms. The average for the 14 birds of her own fraternity and ancestry was therefore only 149 mgms sugar. This is exactly the value obtained by us for ring doves in general and is the probable value which female P995 would transmit to her offspring.

From the preceding data and discussion it may be concluded that in this second generic cross a sugar value of about 149 was crossed with another of approximately 170. The two groups of hybrids obtained—male and female—are both shown in Figure 3 to have had blood sugar values intermediate to those borne by their two parents. Apparently these values in the offspring are nearer to that of the *Spilopelia* parent; they would be exactly intermediate if the true blood sugar value of *Spilopelia* should prove to be about 178. Whitman (16) has shown that *Spilopelia* has a closer phylogenetic relationship to *Turtur* than to *Streptopelia* and this would lead to an expectation of a higher value than 170 in *Spilopelia* rather than of a lower value. And we have already noted that a single individual of *Spil. chinensis* gave a value of 173. Unquestionably both groups (males and females)

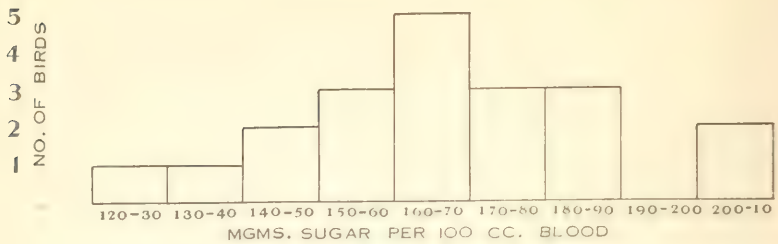

FIGURE 4

Polygon showing distribution of blood sugar values obtained from 20 healthy F_1 *Spilopelia* × *Streptopelia* hybrids.

of these hybrids have values distinctly above the known sugar values of *Streptopelia*; and they have a smaller value than that found for their *Spilopelia* parent.

It is of interest to note that the F_1 hybrids described above have a lower sugar level than have the hybrids from the comparable ♂ *Turtur* × ♀ *Streptopelia* cross shown in Table I— the males there giving 174 against 163 here; the females 169 there against 164 here. The *Turtur* parents of the earlier cross had a sugar value of 188, while the *Spilopelia* parent of the present cross apparently had a sugar value of only 170. Figure 4 supplies the evidence that the average values obtained here do not result from a combination of values from a bimodal curve. The polygon of distribution of the F_1 hybrids is again such as to indicate that the character crossed behaves as do intermediate or blending characters.

We have elsewhere (10) given full consideration to the question whether there is a sex difference in the blood sugar level in males and females of pure species. On the question of such a sex difference in hybrids we possess only the data presented above. The sex differences found in hybrids from the *Turtur* × *Streptopelia* cross are perhaps sufficient, if they stood alone, to suggest a higher value in the male hybrids. But the data for the *Spilopelia* × *Streptopelia* hybrids do not support this view and the point must be regarded as quite uncertain. Do the reciprocal hybrids have equivalent sugar values? On

this matter also our data are all given here and the results from the single group thus tested (*Turtur* × *Streptopelia*) seem to indicate a higher sugar value in the hybrids when *Turtur* is used as the male parent. It is possible, however, that if duplicate determinations had been obtained from the 16 birds with unchecked determinations (second division of Table I) their checked values would have been quite close to those tentatively tabulated for them, and that significant differences for both reciprocal crosses and for sex in hybrids would thus be largely eliminated. Conclusions on these points can not now be drawn; but these possible differences are noted as the reason for using *mean* values instead of averages in dealing with the data of Table I. Fortunately, the mean and the average are almost identical for most of the groups represented in that table.

Discussion

The data of the present paper clearly indicate: (a) That in the two generic crosses made by us the blood sugar of the parent genera stood at distinctly different levels; (b) that the F_1 hybrids from both of these crosses had values intermediate to those of the parent genera; (c) that this intermediate value in the one tested case again divided or "fractionated" in a back-cross with one of the parent species; (d) and that in still other fractional parts of parental composition (five-eighths, one-thirty-second) there is found some evidence that the sugar level is either further divisible or that it still shows the presence and hereditary effect of both parental genera. The data are not of such nature as to test the question as to whether segregation does or does not occur and are otherwise obviously limited in amount and in their application. But the character here studied is a less superficial one than are the visible characters whose hereditary behavior have become well known, and the possibility of an influence of this more deeply-seated character upon those visible and more superficial characters is a point of interest.

TABLE II

APPROXIMATE AND COMPARATIVE AMOUNTS OF REDUCING SUBSTANCES IN THE BLOOD OF VARIOUS ANIMALS AND IN SEVEN GENERA OF PIGEONS

Animal	No. of Animals	No. of Determinations	Reducing power of blood (milligrams sugar per 100 cc blood)	Observer
Man	.....	61	84	Strouse
Horse	.....	2	70	Abderhalden
Sheep	.....	2	70	Abderhalden
Cat	.....	16	70	Scott
Dog	.....	7	75	Scott and Hastings
Rabbit	.....	25	118	Scott and Ford
Duck	.....	14	150	Kausch
Goose	.....	6	150	Kausch
Fowl	.....	9	200	Saito and Katayama
Tortoise	.....	4	90	Bierry and Giaja
Frog	.....	26	40	Scott and Kleitman
Dog-fish	.....	4	65	Scott
Chimaera	.....	.....	28	Lang and Macleod
Cancer	.....	.....	40	Lang and Macleod
Octopus	.....	.....	32	Bierry and Giaja
VARIOUS GENERA OF PIGEONS				
Zenaidura carolinensis	2	5	257	Unpublished
Turtur orientalis	22	43	190	Riddle and Honeywell
Leucosarcia picata	1	5	190	Unpublished
Common pigeon (Columba)	11	22	185	Scott and Honeywell
Common pigeon (Columba)	37	103	181	Unpublished
Spilopelia chinensis	1	3	173	Unpublished
Spilopelia suratensis	1	3	170	Riddle and Honeywell
Stigmatopelia senegalensis	9	15	168	Unpublished
Streptopelia risoria Streptopelia alba	22	36	151	Unpublished

The curve for "body weight" which accompanies the data for blood sugar in Figure 2 is so similar to the curve for the latter as to permit the inference that the mechanism for the inheritance of the one applies also to the other. It must be pointed out that among doves or other animals there is no direct relation between body weight and blood sugar level such as would naturally result in the parallelism seen in Figure 2. The species with the highest blood sugar shown in Table II (*Zenai-*

dura) is with one exception the smallest species represented in the table; the smallest species (*Stigmatopelia*) has, with two exceptions, the lowest sugar, etc. Nor within the limits of a dove species is there any definite relation between the body weight and the blood sugar. The multiple factor hypothesis is the rather widely accepted interpretation of the more usual behavior of "body size" in inheritance. This interpretation is supported in most studies of this subject by one or another amount of observable and measurable segregation. Whether segregation of blood sugar values actually occurs is, as stated above, a question not tested by our data; we may add, however, that we do not therefore assume that it does occur. On this point our own view is that it is fairly clear that the most fundamental characteristics or properties of living matter—irritability, respiration, contractility, growth, etc.—obviously do not segregate, and that a multitude of visible characters certainly do segregate. Whether the normal hereditary level of blood sugar goes with the one group or with the other is quite unknown. The relatively large rôle of three to several glands of internal secretion, and of a part of the nervous system, in the normal maintenance and regulation of the blood sugar level would seem, however, to afford a logical basis for the assumption that the blood sugar level may have a "multiple factor" basis and that some or many of these factors may be segregable.

A few available observations bear in some degree on the point just mentioned. Pike and Scott (8) have called attention to the fact that a mechanism for maintaining the constancy of the sugar of the blood is one of a number of internal adjustments which the progressively higher animals have had to develop in greater and greater perfection. And Scott (14) has further suggested that in higher animals a delicate secondary mechanism has been developed and passed on through inheritance which permits a rapid variation of the blood sugar about the species norm as an emergency means of

securing a prompt release of energy. All of these considerations emphasize the fact that the sugar of the blood is both a regulated and a regulating mechanism; and that its changes—whether of permanent or temporary nature—are of much greater significance to cells and tissues generally than are changes in the ordinary visible characters with which our studies of inheritance commonly deal.

It was noted at the beginning of this paper that studies on the inheritance of characters comparable with blood sugar values have not been made.[2] In this connection it should perhaps be remarked that the behavior of starchy and sugary characters—which are really alternative forms of the same thing—have received much attention in plant genetics. In maize crosses, for example, the chief facts for starchy and sugary endosperm have been reported by Correns (1), East and Hayes (2) and others. A paper by Pearl and Bartlett (7) supplies quantitative determinations of starch, various sugars, moisture, etc., in P_1, F_1 and F_2, from a one-direction cross made with two varieties of corn. In all the above work with maize a segregation of these sugary and starchy characters is shown to occur in the F_2. The F_1 endosperm of corn is of course not wholly comparable with the F_1 of the corn plant nor of the F_1 animal organism. The results of none of these studies seem really comparable with a character such as the blood sugar which, in the animal body, is under continuous regulation and whose temporary or permanent fluctuation in the circulating fluid calls forth other equilibrium changes in both the blood and in all tissues supplied with blood. That such equilibrium changes or adjustments on the part of blood and tissues accompany the permanently high or low sugar level follows necessarily from the general physico-chemical rela-

[2] Since 1920 our colleague, Dr. J. Arthur Harris, has been conducting extensive investigations on the physico-chemical properties of the tissue fluids of Egyptian and Upland cotton, and of their hybrids, as grown under irrigation agriculture at the Cooperative Testing Station, U. S. Department of Agriculture, at Sacaton, Arizona.

tions involved. But a special consideration of some aspects of these adjustments to altered concentrations of the blood sugar has already been supplied by Epstein (3) and by Woodyatt (13, 17).

The blood change found and described by Gerould (4) in blue-green caterpillars is a quite different matter from the subject under consideration but may be briefly mentioned here. In this case the mutational character involved the destruction or decolorization of xanthophyll in its passage through the intestinal epithelium. As a result of this destruction the caterpillar's blood was free of the pigment, and all the various parts of the animal's body which were normally pigmented with xanthophyll through the blood supply were left without this pigment in all stages of the life-history of the insect.

The foregoing results lead to the suggestion that the intermediate blending or divisible nature of such characters as the blood sugar level in the F_1, and a second divisibility in the F_1 back-crossed to a parent genus, affords a possible explanation of some observed irregularities in the behavior of visible characters in wide crosses as this has been observed particularly by Whitman (15). Wide differences of such a character as blood sugar may usually be expected only in forms rather widely separated phylogenetically. That this is true in the case of blood sugar itself is indicated by the data assembled in Table II. From crosses of different genera of pigeons—where fertility extends even to different families—Whitman observed a number of phenomena which it is of interest to review in the light of the facts given above.

The final chapter of the second volume of Whitman's studies is devoted to a consideration of "The divisibility of characters." It was there shown that the F_1 obtained from crosses of wild species and genera of pigeons exhibit (sex limited characters partially or largely excepted) a blending of the parental characters. Moreover, the back-crosses of these F_1 hybrids again show a second

or even a third "division" of the parental characters. Involved in these cases were such characters as general body coloration, the "neck-mark" as a whole, and both the coloration and bifid condition of the individual feathers within this neck-mark. We may note here that the analysis of the divisibility of the blood sugar level observed by us in the *Turtur* × *Streptopelia* cross quite closely parallels the behavior of the visible characters of these same hybrids as studied by Whitman. Again, our second case (*Spilopelia* × *Streptopelia*) involves the identical cross in which Whitman has described (15; see plate 34) a second "division" of the three above-mentioned characters of the neck-mark. Hereditary behavior of the above kind Whitman called "pure or direct inheritance."

A second type of irregularity of hereditary behavior encountered in these crosses of different genera by Whitman is concerned with the impurity or incomplete dominance or recessiveness of characters, notably of color characters; this being sometimes found even in cases of sex-limited color inheritance. For example,—in the very same birds used as principal material for the present study—the white color of *Streptopelia alba* never emerges even approximately pure white from crosses with *Turtur,* but shows various areas of partial pigmentation. These cases could also of course be "described" in terms of multiple factors, modifying factors, contamination, etc. The possibility that unequal numbers of chromosomes are contributed by these genera might also be considered. The results of this study, however, raise the question: Are the new and different levels of blood sugar—and of other similar though as yet unstudied elements introduced into the hybrid by wide crosses—perhaps these modifying or contaminating factors? The new and different blood sugar level of the hybrid certainly provides a somewhat different internal environment in which the characters proceeding from the parent forms must develop. Is no mark left upon the

multitude of visible characters which must undergo the whole of their development under this new, persistent and intimate environment?

Still a third large group of cases of aberrant developmental or hereditary behavior is encountered among the hybrids proceeding from many wide crosses. The exhibition of fertility in all degrees and the fixed or limited life-terms of offspring in some of these crosses constitute one group of such characters; and the greatly increased frequency of reproductive abnormalities and of absent gonads in these offspring supplies us with another.

In none of these cases cited above is it necessary to suppose that an altered sugar level alone, nor that an alteration of any other single one of the several invisible characters which probably share the blood sugar mode of inheritance, is the sole cause of the aberrant behavior. The important point would seem to be that a new internal (blood) environment is established in such hybrids and that the parental characters show a developmental response to this new environment. We have here been able to show that one important element of such a new blood environment does occur in some of these hybrids; and we have noted that some of the characters of these same hybrids show atypical or unexpected hereditary behavior. Elsewhere we have shown (10) that the normal sugar level extends throughout the life cycle in the pigeon, and is already present in the embryo.

At the beginning of this paper it was noted that from some reported crosses of species or of genera there result, apparently, no notable departures from expected hereditary behavior. We are now able to offer a possible explanation for the issuance of normal hereditary behavior from certain wide crosses and abnormal or unexpected behavior from certain others. For, in some such crosses the blood sugar level—and other similar regulating characteristics of the blood or internal environment—may not be notably different in the forms crossed:

while other crosses do involve wide differences in these characteristics of the internal environment. In these latter cases the continued "fractionation" of such characteristics of the internal environment assists in the continued blending or fractionation of characters in the hybrids of the various grades. It is thus made evident that in the case of some wide crosses an agency for the modification of developing characteristics may come into action which is necessarily absent from crosses of closely related races, varieties and species.

On the basis of the facts obtained from the present study of the blood sugar of dove hybrids it is reasonable to assume that there exist other as yet unstudied characteristics of the blood—or internal environment—which behave in inheritance in the same way as does the blood sugar level. Some of these characteristics, states or processes may affect developmental processes more readily than do others. We venture the suggestion that some or many of the visible characteristics of two parent species which have developed in, and are presumably perfectly adapted to, a blood environment at one level may exhibit a response of one or another grade when forced to develop in a different blood environment in the soma of the hybrid. If this is true we are in a more favorable position to interpret some of the hereditary and developmental phenomena presented in hybrids from wide crosses.

SUMMARY

The genera *Turtur* and *Streptopelia* were observed to have quite different amounts of sugar in the blood—respectively 188 and 149 mgms sugar per 100 cc of blood—and a study was made of the amount of blood sugar in various grades of hybrids of these two genera. A study of F_1 hybrids of a *Spilopelia* × *Streptopelia* cross was also made.

The F_1 hybrids obtained from both crosses had blood sugar values intermediate to those of the parent genera.

The offspring of F_1 hybrids of *Turtur* × *Streptopelia* back-crossed to *Streptopelia* again gave sugar values intermediate to those of the F_1 and *Streptopelia*. Still other grades of these hybrids obtained from back-crosses apparently also show a further blending or "fractionation" of this character.

The blood sugar level is apparently inherited in the same way as is body size in these same animals.

The behavior in inheritance of a character comparable with the normal blood sugar level has hitherto not been studied. The hybrids examined had a blood sugar value different from the values of the parent genera; this different value is maintained throughout the life cycle of the hybrid; and this different sugar value requires equilibrium adjustments on the part of other blood and tissue components of the hybrid in all or nearly all stages of the development, growth and life of the hybrid.

It is suggested that certain irregular and other little known hereditary or developmental phenomena often met with in hybrids proceeding from wide crosses are the result of the circumstance that in such hybrids the parental characters are forced to undergo the whole of their development in blood which constitutes a new, intimate and continuously acting internal environment.

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THE EFFECT OF THE ENVIRONMENT IN THE PRODUCTION OF MALFORMATIONS

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The Effect of the Environment in the Production of Malformations [1]

The mental attitude of the older systematists who regarded all variations as devices of the devil seems to have persisted longer with reference to the various malformations occurring in vertebrates, and its latest efflorescence is apparently found in the studies by some psycho-analysts, and even pseudo-endocrinologists, of the minds of men of genius. I refer to the attempts of two of them to show how Darwin became that way mentally. If I knew how Darwin did get his peculiar mental characteristics, I, for one, would obey the injunction, "Go thou and do likewise." The point of the matter seems to be that the biologist should not wonder so much why there are slight variations or departures from strict conventionality as that there is so little departure. The uniformity of results in such a marvelously complex system as the living organism, rather than the occasional departures from strict tradition, seem most difficult to understand.

This regulation of body form has, as I am inclined to believe, a much more significant relation to such great biological questions as the failure of parthenogenesis and the occurrence of death in higher organisms than has generally been recognized, and all these questions are apparently related in some way to the increasing degree of independence of the external environment acquired by the organism in the course of evolution.

The egg of each species has its own specific rate of development, probably dependent upon its specific chemical make-up, and not upon whether it is holoblastic or meroblastic. The rate of development depends, particularly during the later stages, on the amount of food available, but the supply of oxygen and the temperature have a more striking influence on the rate of development. The oxygen supply and the temperature are the most important and most common influences in the environment which affect the rate of development.

Two methods of development occur in the animal and plant series—the continuous and the discontinuous. The continuous mode of development is found in most of the animals whose eggs develop in a uniform or homogeneous medium, such as sea water.

The fresh-water and the land animals manifest most varied and complex methods of securing an environment of sufficient uniformity to permit un-

[1] Stockard, Charles R., "Developmental Rate and Structural Expression: An Experimental Study of Twins, 'Double Monsters' and Single Deformities, and the Interaction among Embryonic Organs during their Origin and Development," American Journal of Anatomy, xxviii, pp. 115–277, 1921.

interrupted development. Many forms have evolved a method for the development of the embryo within the body of the mother. Not all land animals have succeeded in securing continuous development, and there are many forms in which discontinuous development obtains. In oviparous forms the egg attains a certain stage within the body of the mother, and then, in some species, a break occurs in the environment, with a consequent slowing or stopping of the whole process of development.

A continuous mode of development may be transformed to a discontinuous one by reducing the temperature at any given stage, by restricting the supply of oxygen, or increasing the concentration of carbon dioxide in the environment of the developing egg.

The author's experimental work, as described in the present paper, was done on eggs of Fundulus (the mud minnow), and the particular changes produced in the environment were reduction of the temperature or of the supply of oxygen.

Fundulus eggs show stages of comparative indifference to the slowing of the developmental rate, and other stages in which there is a much greater sensitiveness. If the slowing were to occur from natural causes at the sensitive periods, the species might be eliminated because of the high proportion of abnormal embryos which would result.

If Fundulus eggs are reduced to a temperature of 5° C. to 9° C. immediately before the first cleavage for varying periods—45 hours at 5° C. to 70 hours at 7° C. or 9° C.—and then restored to room temperature (July at Woods Hole), there is a high mortality, and a high percentage—about 33 per cent—of deformities among those embryos which develop further. At a temperature of 5° C. development is almost completely stopped, and the mortality among the eggs subjected to this temperature, when again brought to room temperature, may be as great as 78 per cent. After exposure to temperatures of 7° C. or 9° C., by which development is greatly retarded, the mortality may be 54 per cent or even only 34 per cent. The monsters resulting are not limited to any one type, but almost all types may be exhibited. Stopping the development almost completely has a more severe effect upon the embryo than merely slowing it down.

When, however, development has proceeded through gastrulation and the appearance of the embryonic shield, subjecting the eggs to the low temperatures for three or four days has but relatively little effect beyond deferring the time of hatching. This is true also when the eggs have attained even later stages of development before they are subjected to lower temperatures. In the case of Fundulus, the early cleavage stages seem particularly sensitive to environmental conditions which slow the rate of growth, while the later stages seem insensitive to such conditions.

The reason for the appearance of the great variety of monsters after the early cleavage stages have been subjected to the low temperatures is that the

development of all organs must occur subsequent to this, and that all may thus be arrested or deformed. No double monsters occur if slowing of development occurs at the beginning of gastrulation, as their moment has passed, although various brain, branchial, and other defects may appear.

The types of deformities resulting from stopping or slowing the rate of development at critical periods are the same as those resulting from treatment of the eggs with solutions of various chemicals. Reduction of the oxygen supply by allowing the eggs to form clumps of 100 or more was attended by the same general effects upon the eggs in the center of the clump as were noticed after a reduction of the temperature.

Trout eggs are more sensitive to a reduction of the temperature or to a lack of oxygen than Fundulus eggs. Twins and double monsters were more frequent in the trout eggs so treated. Stockard concludes that twinning and formation of double monsters are not due simply and solely to changes in the environment, but are also dependent upon the internal structure of a given egg and its peculiar manner of development.

In connection with this greater sensitiveness of trout eggs, it would be of interest to determine the temperature and the oxygen content of the water in the streams where these fish spawn, and in other streams, at the critical period of the development of the eggs. Such a study might have some bearing on the question of the distribution of the trout and of other fish.

Twins and double embryos are relatively frequent in fowl's eggs, but these rarely hatch. Birds have a discontinuous mode of development, with a slowing or arrest often occurring within a short time after the egg is laid. But the blastoderm has usually passed the gastrula stage at the time of laying. There is probably, however, considerable variation in the stage of development at the time of laying, and many eggs have probably failed to reach or pass the gastrula stage. Stockard considers that this variation is sufficient to account for the occurrence of double individuals and twins among chick embryos. There appears to be a selective factor in the time of laying. Eggs laid before the stage of gastrulation has been reached would, on the basis of the experiments herein described, be particularly susceptible to the slowing of development following laying, and the relatively large proportion of abnormalities would result in the virtual elimination of these eggs, and perhaps of all the eggs laid by a particular mother.

The egg-laying reptiles, since their body temperatures is only slightly higher than that of the environment, are not subject in the same degree as birds to the dangers of a discontinuous mode of development. So far as birds are concerned, the discontinuous mode of development has arisen from the fact that birds have developed a uniform body temperature. It would be of some biological interest, from the point of view of the selective time of laying, to determine whether the stage of the development of the avian egg at the time of laying differs from that of the reptilian egg. Another matter

of some interest, but beside the point of the origin of the malformations, would be the collection of data bearing on the total time of development of the young in oviparous and viviparous types of the same phylum, as in fishes and reptiles, under the same conditions of the environment. One is struck by the relatively short period of incubation of birds as compared with the period of gestation of most mammals.

The attempt is made to explain polyembryony in the armadillo on the basis of a period of quiescence in the developing egg and a probable lack of oxygen because of the imperfect uterine implantation of the egg during this stage. There is a period of quiescence during the development of the dog's ovum, but it is unknown at what stage it occurs, and it is equally unknown why polyembryony does not occur.

The argument is advanced that the adult body of higher animals may be considered to arise from a sexually produced embryonic axis, the stock of which gives rise by asexual budding to the various special organs. This constitutes an alternation of generations—a process not limited to lower forms, but occurring throughout the animal kingdom.

This consideration has a bearing on the relation between structural components of connected twins and double individuals. There arises a competition between the components themselves similar to that among several buds growing from a common stock. This is reminiscent of Roux's "Kampf der Teile." [2]

In double individuals, the parts of which are incompletely separated, there may be all variations from equality in the size of the two parts to relative insignificance of one, so that it appears as a mere bud on the other. When the two parts are of equal size, both may be—and generally are—structurally perfect. In the competition between the parts the advantages were apparently equal. Where the two components are unequal in size, the smaller component is always abnormal in structure. The greater the difference in size, the greater in general is the degree of abnormality of the smaller component. There is a possibility that the smaller component may be entirely included within the body of the larger.

From this point of view, an individual appearing to be a typically single specimen may in reality partake of the nature and qualities of the larger component of a double individual. Since reversal of the viscera occurs much more frequently in one of the components of a double individual (rabbit) than is ever found in any group of single vertebrate specimens, the reversed arrangement of the viscera would appear to be associated with the double condition; one may be inclined to suspect that a typically single individual with *situs viscerum inversus* is in reality a twin—*i.e.*, the larger component of a double individual.

[2] Roux, Wilhelm, "Die züchtende Kampf der Theile oder die 'Theilauslese' im Organismus," Abhandlungen über Entwickelungsmechanik der Organismen, Bd. I, pp. 135–422, 1895.

The condition of the small component has a bearing also on certain theories of teratomata. Stockard argues that a teratoma may represent simply the smaller component of a double individual.

The two components may sometimes be separated completely and give rise to identical twins from one ovum.

In the double individual with unequal components the question arises as to the influences which could act upon the smaller component to interfere with its complete development, but which could not also act upon the larger. Agencies in the external environment must be excluded, since they would act equally upon both components because of their intimate connection and inclusion within the same egg membrane. Nor can the defects in the smaller component be, in general, of germinal origin. The question of the competition between the various parts of the organism is again raised, and the conclusion is drawn that this competition must be something other than that for the appropriation of the ordinary yolk material. It may be similar in nature to that between two or more plant buds growing from a common stock, when the competition involves something more than a struggle for the available food. In some manner, not at present understood, the larger component of a double individual has some advantage over the smaller, and so develops normally, while the smaller component is malformed.

In summing up the evidence, the author states that every type of developmental monster may be produced by one and the same experimental treatment; the same structural abnormality may be induced in the embryos of various species by a great number of experimental treatments; in all cases the initial effect of the experimental treatment is a lowering of the developmental rate, and the resulting deformity is always due to this slow rate of development; the type of monster or deformity is determined by the developmental period during which the slowing in rate is experienced. As a common law of both abnormal and normal development, we may say that "structural quality may be affected by many things, but always depends directly upon the rate of development of the part of the individual."

A particular structural modification may be more common in one species than another. Double individuals are more common in trout than in Fundulus, but some eye defects are more common in deformed specimens of Fundulus. These conditions are apparently due to the different hereditary backgrounds on which the modifying conditions act.

It is possible that the early formed organs in a single individual may be related to one another in their development as are the components of double individuals. The examination of the growth influence of the apical or primary bud over the secondary and potential buds in plants is particularly suggestive in this connection. The general conclusion is that each growing bud and each embryonic organ exerts a depressing influence on the growth of all other buds in the individual plant. As a shoot gradually ceases to grow,

its depressing influence gradually ceases. One may wonder whether the last sentence applies universally in animals.

In the interactions of the various organs of the embryo with one another we find, at certain intervals of time, rapid growth occurring in given regions. The growth in certain regions then slows down and growth in other regions begins, to slow down in its turn. In the case of the brain of vertebrates, it is possible that during this period of rapid growth the optic vesicles exert a depressing influence over other parts. It is known, too, that the optic vesicles arise from a definitely located group of cells in the neural plate of the embryo, and that they can not arise from any other cells. Furthermore, if the optic vesicles do not grow out at a particular time, they do not grow out at all. There is in this a significant emphasis of the importance of the time factor, long recognized by biologists. We may recall the old statement of Vrolik that many abnormalities of development could not be due to maternal impressions, since the time of occurrence of the event supposed to bear a causal relation to the abnormality seldom corresponded with the time at which the particular organ affected arose or reached a certain stage of development in the embryo.

With reference to the author's question whether there is a competition between lateral and linear growths in other systems, similar to that in the nervous systems, or whether there is any wider competition among the different systems of the embryo, we may recall Burdon-Sanderson's[3] remark that the organisms must have control of all its parts. And Roux's "Kampf der Teile" again comes to mind. A consideration of the effects of a decreased food supply will show that this competition can not be a competition for food alone. Warren has shown that Drosophila larvae, reared on an insufficient supply of food, are smaller than others, but otherwise apparently perfectly formed, and the females are capable of laying as large eggs as flies of normal size.

Stockard finds that in the alimentary tract there is a process of linear growth followed by a process of lateral growth in the nervous system. Abnormalities in the alimentary tract may be produced by arresting the development at the appropriate time, just as in the case of the nervous system. He is inclined to believe that all organs arise as a series of lateral outgrowths or buds which bear a relationship to one another similar to that among buds in a growing plant. One bud is dominant for a time, at the close of which it exhausts its advantage. Some other bud thereupon begins to grow, until it in turn exhausts its advantage. If the whole embryo is depressed or has its developmental rate slowed at a moment when a given bud is growing at its most rapid rate, that bud is more decidedly reduced in its rate than any other portion of the embryo. When the whole embryo resumes a more rapid rate, the slower growing parts are able to resume their ordinary rates, but

[3] Burdon-Sanderson, J., Nature 48: pp. 465, 467, 1893.

the particular bud most affected may have passed its moment of greatest advantage, or may be unable to express itself, since other buds now arrive at the stage of advantage.

The problem is to locate the critical moment for each of the developing organs. By slowing development during a period which will cover a given critical moment, one may be able to suppress any given organ at will. The complexity of animals renders the production of such localized abnormalities difficult with present methods of experiment.

Conditions in plants are simpler, and it is possible to suppress or bring out a given bud at will. Stockard expresses the opinion that, despite the fact that we do not know exactly how it is accomplished, it is definitely the growth of one bud in a plant that prevents the growth of another one. And similarly, in the embryo, probably the growth of a given organ holds back the initial growth of another organ until the first has exhausted its power of suppression. The components of a double individual react on one another in such a way as to suggest this interpretation.

One may venture a suggestion here that the conception of cyclical changes in the cells arising from a given portion of the embryo or those constituting a given bud would obviate the necessity of postulating a depressing influence of one part of an animal embryo upon all other parts. It seems clear that, although some nerve cells retain the ability to undergo karyokinesis up to the age of 25 years in the human (Donaldson),[4] others never divide after birth. Do nerve cells cease to divide because of the development of other parts of the organism, or because of some conditions arising in the nervous system itself? There is in this long-continued life of a nerve cell, after undergoing its last division, a striking analogy to Uroleptis, which may continue to live for a considerable time after losing the power of division (Calkins). Since Uroleptis is a protozoan, one can scarcely postulate the depressing influence of other parts of the organism upon it. Again, many plants have an indeterminate growth, while all higher animals have a determinate growth, associated, as it appears to me, with the regulation of body form. Can we transfer without reservation, and without taking account of this determinate growth, conclusions drawn from observations on plants to animals?

Returning to the question of critical movements in the developmental process, Stockard has succeeded in locating the following moments:

1. The growth of the primary embryonic axis. The critical moment for the origin of twins or double individuals lies in the early cleavage or pregastrula stages.

2. The suppression of the eyes or modification of their structure. The critical moment lies somewhat later than the first, but before the origin of the embryonic shield of Fundulus.

3. Suppression of the primary brain ventricles, leading to deformed or

[4] Donaldson, H. H., "The Harvey Lectures for 1916–1917," Philadelphia, 1918.

tubular brains. The critical moment is before or at the time of the appearance of the embryonic shield.

4. Modified or contorted mouth and branchial systems. The critical moment is during the formation of the embryonic shield or earlier.

There is probably some overlapping of critical moments for the origin of ectodermal organs, as those of the central nervous system, and the entodermic organs of the alimentary canal.

5. Modification in size and structure of the inner ear. The critical moment lies close to that of 4.

6. Faulty development of the liver and the pancreas. The critical moment is after the embryonic line is distinctly visible in Fundulus embryos.

Each organ arises at a definite moment during embryonic development and not during widely different moments just as truly as an individual organ arises from a very definite anlage and from no other.

The relations between the rate of growth and particular developmental moments probably continue to be of importance in post-natal development. One can show a continuity in the series from *monstra in defectu* through normal single individuals to *monstra in excessu* and to identical twins in an orderly series. And it is probable that arrests at certain critical stages of development underlie the appearance of all types of monsters.

The sensitiveness of the developing embryo to various changes in the environment introduces great hazards during this period. Variations of form, often serious in their consequences for the life of the embryo, appear as the result of the action of the changes in the environment at the critical moments of development. Regarded from this point of view, the constancy of animal and plant forms, rather than their variability, is cause for wonder. The variations appearing as the result of the action of the environment, in so far as they are defects or deformities, can hardly be regarded as progressive, and hence can not be regarded as factors, except from the point of view of their selective elimination, in organic evolution. The prevention of these developmental arrests becomes of increasing importance in higher animals. Along with the regulation of internal conditions for other processes or functions there occurs the regulation of the environment of the developing embryo. The control of the environment of the embryo must be included in the list of significant things accomplished for purposes of evolution through the constancy of internal conditions of the organism. One may remark here that the ability of the eggs of marine and aquatic forms to develop so constantly in an environment subject to changes may be considered as an adaptation. As Goodrich points out, regulation of the simplest organisms or of the earliest formed compounds on the road to living matter conferred an advantage, and a power of adaptation sufficient to compensate for any except the extreme variations in the external environment would give the eggs possessing it an advantage over those which did not.

In the final section of the paper the author considers the relation of cancerous growths to the general cessation of all normal growth in the old individual. In age, growth is largely confined to the repair of natural loss and the maintenance of the state of adult balance. Regenerative growth following injuries to the adult animal may become morbid in degree and without regulation, thus giving rise to malignant conditions. Cancer would be expected to be more frequent in certain families since the growth balance and proportions are hereditary characters, and the malignant growth depends largely on these. The slight amount of general growth in old individuals can depress but slightly any regenerative processes that may be set up. On the basis of the general theory, it might be supposed that every mammal would develop some form of malignant growth if it should live to extreme old age.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XV. Increased Blood Sugar Coincident with Ovulation in Various Kinds of Pigeons

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In earlier studies by one of us (1), (2) it has been shown that the suprarenal glands of the pigeon undergo a marked hypertrophy coincident with ovulation. This hypertrophy begins about 108 hours before the next prospective ovulation, and is also quite coincident with the establishment of a new and much increased rate of growth in the ovum next to be ovulated; and is further coincident with the beginning of a remarkable growth or hypertrophy of the oviduct. Since enlargement in these three distinct organs apparently begins at the same time, it has thus far proved impossible to obtain evidence as to which, if either, of these is primarily responsible for this series of interrelated events.

The fact of suprarenal hypertrophy raises the question whether the hypertrophy is shared by both the medulla and cortex and whether each or only one of these parts actually acquires increased functional power. Since adrenalin is secreted by the medulla, and an increase of adrenalin in the blood results in an increase in the amount of sugar in the blood, it is possible to learn from a study of the blood sugar curve whether an increased action of the medulla probably occurs in connection with the enlargement of the gland. Adrenalin is of course only one of the factors which determine the general level of glucose in the blood; but perhaps the "ovulation period" may provisionally be regarded as an "emergency" in Cannon's sense, and thus a period during which an increased output of adrenalin sustains a higher level of sugar in the blood. Whatever may be the relation of the adrenalin output to the amount of blood sugar present during these recurring periods, it was thought of importance to make measurements of the blood sugar during the ovulation cycle which is a cycle accompanied by suprarenal enlargement. It is the result of this particular study which is reported here.

Any metabolic change, of any nature, which is repeated with each ovulation cycle in the pigeon is of much importance to central problems of sex in addition to its bearing on reproduction. For it has earlier been shown by Whitman (3) and Riddle (4) that rapid and continuous ovulation in the pigeon results in a profound modification of the sex ratio (and of sex itself, according to Riddle), and ultimately on longevity and viability of the offspring. Other studies by Riddle have identified these sex and developmental changes or transformations with metabolic changes in the ova and in the female parent producing the ova. As a result of the present study it becomes clear that a pigeon forced to continuous egg-laying is a bird in which the sugar metabolism of the parent bird is almost continuously disturbed and held at an abnormal level. Whether this disturbance signifies an increase or a decrease in the rate of the burning of carbohydrate is a question essentially untouched by this study; but the fact that a change or modification in the carbohydrate metabolism occurs is established.

An account has elsewhere been given (5) of one of three series of determinations made in order to learn whether the blood sugar increases at ovulation in a way at all smilar or parallel with the increased weight of the suprarenal gland. In the present paper all of the data bearing on this subject as obtained in three series of determinations will be presented. The only previous work in which an association of high blood sugar with any aspect of egg production has been suggested was that of Scott and Kleitman (6) in studies on the frog's blood. These authors concluded that during the months of February, March and April the "sugar content of the blood of males is lower than that of females, and it is lower in the females with poorly developed eggs than in those with well filled ovaries." These authors further state that their results "indicate that this condition (value of the blood sugar) is directly related to the condition of the eggs." It will be seen that the present study definitely relates the increased sugar values to their proximity to ovulation.

Methods. *The care and handling of the birds.* All birds were kept with their male mates in well-lighted pens of adequate size and under such other conditions as have been demonstrated to permit practically continuous egg-laying throughout the year, and from year to year. Accurate egg-laying records are kept for all of the birds of the whole collection from which the birds used in this study were chosen. The birds used were not permitted to incubate after laying, but the males were sometimes removed at the time of one ovulation period in order to prevent the almost immediate resumption of a new cycle of growth in ova, oviduct and suprarenals

The common pigeons and the hybrids used were not particularly tame birds. Before any blood samples were drawn from them these birds were therefore caught and handled by the operator, and kept close at hand during a preliminary period in order to minimize danger of a rise in blood sugar due to fright when samples of blood sugar should be drawn. The ring doves were quite tame, were accustomed to handling, and did not seem to require the above precautions. All birds were caught and brought into the laboratory where they were kept in very small cages for at least two hours before samples were drawn. Two samples from the same bird were never drawn at less than a two-hour interval, nor in less than two hours after the last previous handling of the bird for any purpose. The "scragglies" used are a mutational form of common pigeon which shows conspicuous epidermal anomalies, and are separately classified here because they have consistently shown lower values of blood sugar than do ordinary common pigeons.

Special methods and technique. Maclean's micro-method was used and duplicate determinations were usually made on each ovulation stage studied. For the few cases in which these determinations did not check to within 10 mgm., additional determinations were made in most cases. In order to obtain twice or three times an exactly equivalent ovulation stage the determinations made upon a single bird (series I) were sometimes carried out over a period of three or four weeks. Details concerning the time interval (44 hours) between the ovulation (mid-ovulation) of the first and second eggs of the pair produced by doves and pigeons, and also the data concerning the period of beginning suprarenal hypertrophy and of the growth of the ovum, may be found in the preceding paper of this series (2).

The samples were drawn by needle-puncture of the heart. This occasionally resulted in the death of the bird. While the samples were being drawn the bird was encased in a close-fitting cloth jacket made for this purpose. Other details of the method followed here are those earlier used and described by Honeywell (7). In series I we obtained data for three or four different periods in the ovulation cycle upon the same individual bird; the several birds of the group therefore supply data for a still larger number of periods in the cycle (see curve). This method of obtaining these particular samples required of course that the bird be not killed after drawing the earlier samples and here it was impossible to verify an expected pre-ovulation stage at autopsy. The autopsy was usually unnecessary for a definite fixation of the mid-ovulation stage and for the three or four days preceding this stage if a succeeding ovulation occurred in less than four days thereafter. Near the close of this first series of determinations it was learned, however, that the needle-puncture of the heart occasionally *suppresses* the

ovulation of ova which are very nearly ready to ovulate and causes their regression in the ovary. In other rare cases the heart-puncture, or the heart-puncture and associated handling and treatment, resulted in the passage of the ovum into the body cavity instead of into the oviduct. In the light of these facts, and of our later results in which we were able to exclude all uncertainty, it is wholly probable that data for one period, as obtained from one of the common pigeons and from one of the "scragglies" used in series I, were not properly classified.

Presentation of data. The main results of the investigation are sufficiently shown in a condensed form in table 2 and on the curves of figure 1. A more detailed presentation of the results from series I has been given elsewhere (5) and the details of series II (hybrids) are adequately shown by the curve. Table 1 fully presents the results from series III, which was solely concerned with the difference of sugar values measured at mid-ovulation (i.e., after the ovulation of the "first," and before the ovulation of the "second" egg) and at another point removed from ovulation at which the bird was killed so as to fix the ovulation stage beyond doubt. The results obtained from this series are highly consistent and leave no doubt that in these two groups of birds blood sugar was distinctly greater at the ovulation period than at points separated by more than 48 hours from it.

The summary given in table 2 shows that in all of the four kinds of birds, and in all of the six groups of birds studied, there is a greater amount of sugar in the blood at the period of ovulation—i.e., within the interval which elapses between the separation of the two ova from the ovary—than at points more distant than 36 hours from the beginning or end of that period. The individual exceptions within the groups are very few in number, and are probably the result of uncertain identifications of the periods, particularly in series I. Moreover, the sugar values obtained for the six or seven additional groups of birds for whom determinations were made only at mid-ovulation, or only at a point removed from the ovulation period, are in all cases (considered as groups) of a magnitude corresponding to that of their respective periods. The averages of the four kinds of birds studied in all of the three series are all in the same sense. The mid-ovulation stage and stages more than 36 hours from ovulation respectively, are: For common pigeons, 208 and 183 mgm. per 100 cc. of blood; for scragglies, 186 and 160; for ring doves, 176 and 149; for hybrids, 213 and 171. The mean values obtained for all birds in all groups are 196 and 166. The mean values obtained for 32 individual birds whose sugar was determined in both divisions of the ovulation cycle are 194 and 167. It is clear that all groups of birds show considerably increased blood sugar values at the period of ovulation. Mid-ovulation values compared with values ob-

TABLE 1

Details of the blood sugar determinations of series III

	BIRD NUMBER	STAGE IN RELATION TO OVULATION AND AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)		
		Before or after ovulation (hours)		Mid-ovulation period
		108-49	48-0	
Common pigeons	1	185		(205)*
	2	183		203
	3	178		193
	4	178		198
	5	173		198
	Average	179		199
	6	175		
	7	183		
	8	183		
	9	195		
	10	(195)		
	Average	186		
Ring doves	1	153		198
	2	115	123	(140)
	3	170		180
	4	155		(160)
	5	153		(150)
	6	143		170
	7	163		188
	8	163		180
	9	160		193
	10	148		193
	11	163		188
	Average	153	123	176
	12	155		
	13			190
	14			(155)
	15			(195)
	16			(185)
	Average	155		181

* Parentheses enclose single unchecked determinations.

tained from 108 hours before and 108 hours after ovulation indicate that the blood sugar at ovulation is increased by rather more than 20 per cent.

TABLE 2

Amounts of blood sugar obtained within, and apart from, ovulation periods in various kinds of birds

SERIES	KIND OF PIGEON	NUMBER OF BIRDS	PERIOD AS RELATED TO OVULATION AND AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD	
			Mid-ovulation	36 + hours before or after ovulation
I	Common pigeons	4	216	185
		1	220	
	Scragglies	4	186	160
		1		120*
	Ring doves	6	172	140
		1	185	
		2		158*
II	Alba-orientalis hybrids	2	213	185
		3		161
III	Common pigeons	5	199	179
		5		186
	Ring doves	11	176	153
		4	181	
		1		155
Average	Common pigeons	10	208	(14) 183
	Ring doves	22	176	(18) 149
	Scragglies	4	186	(4) 160
	Generic hybrids	2	213	(5) 171
Means, all birds in all groups		38	196	(41) 166
Means, identical birds, both stages		32	194	(32) 167

* Neither mid-ovulation nor more than 36 hours removed from it; classed with neither group in averages.

The curves supply the evidence that there is a gradual rise of the blood sugar value beginning at or near 108 hours before the ovulation period, reaching its maximum in this period, and again undergoing gradual decline. In all these respects the blood sugar curves resemble

the curves for suprarenal hypertrophy earlier described by Riddle (1), (2).

Only in series I and II were data obtained at intervals permitting their arrangement in the form of curves. A curve for the "scragglies" of series I is omitted. It is a less regular curve than those presented, but the mid-ovulation value is higher than any other point in the curve. The data for the "scragglies" are defective. Two of these birds were killed by the first heart-puncture and only unchecked values were obtained. In addition, one bird of this group probably failed to ovulate properly because of heart-puncture in a way similar to that of one common pigeon whose effect on the uppermost curve of figure 1 may next be noted.

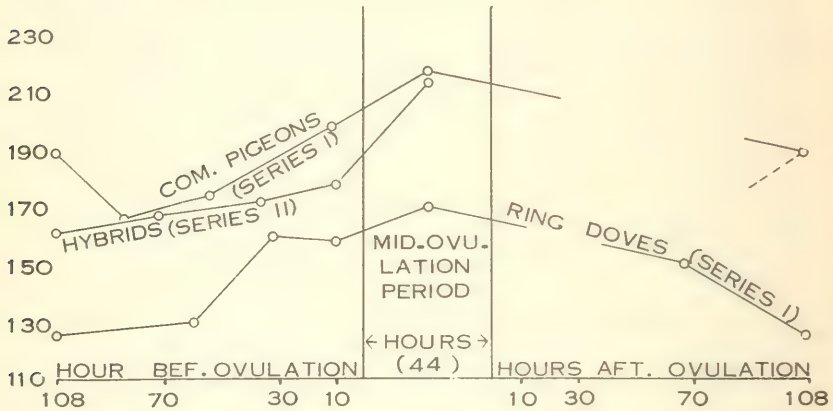

Fig. 1. Curves showing average blood sugar values obtained from three kinds of pigeons during ovulation and at a known number of hours before or after ovulation. The base line marks off time in hours. The ordinates represent milligrams sugar in 100 cc. of blood. For explanation of the ends of the curve for common pigeons see text.

Only three of the five common pigeons used in series I are represented in the extreme ends of the curve (108 hours before and 108 hours after ovulation), and one of these had a very high blood sugar (228 mgm.) which is probably wrongly classified because, as noted above, the correct stage could not at the time be verified by autopsy. This bird was five days removed from the last preceding ovulation when two samples were drawn; after receiving these two heart-punctures she did not again ovulate until another five days had passed. We are thus forced to record these values at both points farthest removed from ovulation though they probably belong to a stage very near to ovulation—an ovulation suppressed by the heart-punctures. The extreme ends of the curve for this group are probably high and irregular for this reason.

The curve for the hybrids (*Streptopelia alba* × *Turtur orientalis*) is given added significance by the circumstance that 31 non-reproducing females, sisters of birds used for curve (but with no ovulation during the previous 60 to 350 days, and therefore not used in the present study), were found to give an average value of 161 mgm. sugar; and from the further fact that the highest value obtained from any of these 31 individuals was 205 mgm., though both of their sisters examined in the mid-ovulation stage showed still higher values.

The curve for ring doves includes the data obtained from the five individuals which at autopsy proved to be free of discoverable disease (tuberculosis, Ascaridia). The curve is less smooth when the diseased birds are included but is otherwise quite similar.

The data for series I were obtained in March; for series II in June to August; for series III in September. A fourth series was attempted in late September and in October but unexpected effects of the onset of cold weather, while our birds were unprotected from the cold, became apparent and it was learned that soon after the beginning of cold weather both the ovulation and non-ovulation blood sugar values are definitely decreased. This effect of cold was therefore further studied, and all results obtained at that period, including partial data for a fourth series of determinations on the present subject, are being prepared for publication.

All of the birds used in this study were killed after, but not in all cases immediately after, the last blood samples were drawn, and the weights of their suprarenals obtained. The notable disturbing effects of disease on suprarenal size, and our failure to find time to kill several of the birds immediately after taking the last blood sample, serve to complicate the data obtained in our attempt to examine this question. Similar attempts to learn whether the thyroids of the bird undergo size-fluctuations coincident with this sustained rise in the blood sugar curve, and whether a relation exists between thyroid size and the non-ovulation blood sugar values of different birds of the same kind, have also proved too complicated for present action here.

SUMMARY

Each ovulation period in the pigeon is accompanied by an increase of approximately 20 per cent in the amount of sugar in the blood.

The sugar content of the blood begins to increase at about 108 hours preceding the ovulation of the first egg; it is then maintained at the highest level until the second ovulation 44 hours later; following this period it probably gradually declines to the normal non-ovulation level in an additional 108 hours—unless new ova meanwhile begin their final period of rapid growth and a new ovulation cycle is begun.

The curve which thus describes blood sugar values as related to ovulation is essentially similar to the curve for suprarenal hypertrophy at ovulation earlier described by Riddle.

The above results apply to various species of doves, pigeons and their hybrids.

Female pigeons forced to rapid and continuous ovulation throughout the year are thus forced to an almost continuous maintenance of their bodily functions—and to the production of their eggs—under altered and unusual conditions as regards their carbohydrate metabolism.

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AN ARTIFICIAL RESPIRATION AND ETHER APPARATUS FOR USE WITH COMPRESSED AIR

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AN ARTIFICIAL RESPIRATION AND ETHER APPARATUS FOR USE WITH COMPRESSED AIR*

By H. F. Pierce, A.B., B.Sc., (Oxon), New York, N. Y.

WHEN compressed air is employed for the administration of artificial respiration the flow of air must be interrupted a certain number of times per minute in order to simulate breathing. This is usually accomplished by means of automatic valves such as have been described by W. T. Porter, W. B. Cannon, and others. These devices, while satisfactory for research, present certain disadvantages when numbers are required for

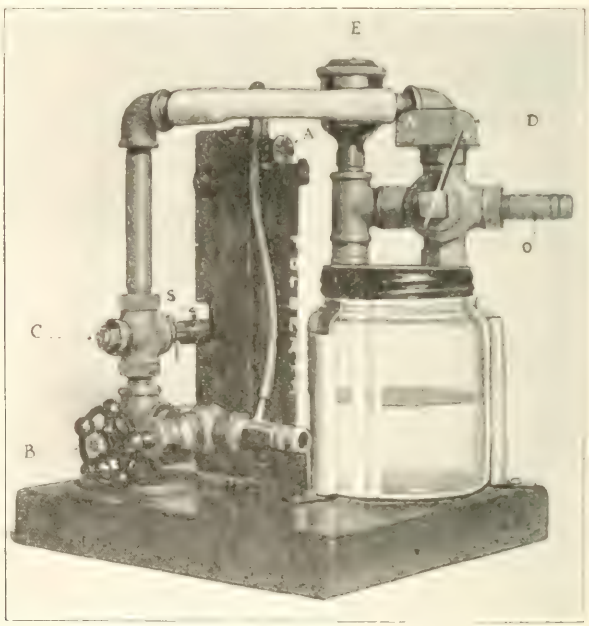

Fig. 1.

student courses. For example, the electric motor driven interrupter is rather expensive, and the tip-bucket valve requires running water to operate it and a sink to carry away the waste.

In the apparatus here described, power for operating the interrupter valve is derived from the compressed air, acting through the agency of a reciprocating air motor similar to those used in automobile windshield wipers.

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Referring to the accompanying photograph, (Fig. 1), compressed air from the mains enters the apparatus at H. A part of the stream is shunted through the small vertical tube to the motor, which is seen in the background at the left. Another part passes through the needle valve B, the interrupter cock C, and the ether apparatus, to the animal.

The speed of the motor, and hence the number of respirations per minute, is controlled by the small knurled screw A. The amount of air passing to the animal is regulated by the needle valve B. By loosening the set screw S and rotating the sleeve on the motor shaft, the period of the inspiratory phase may be lengthened or shortened. By stopping the motor during the inspiratory phase of the cycle a steady stream of air may be obtained for tracheal insufflation.

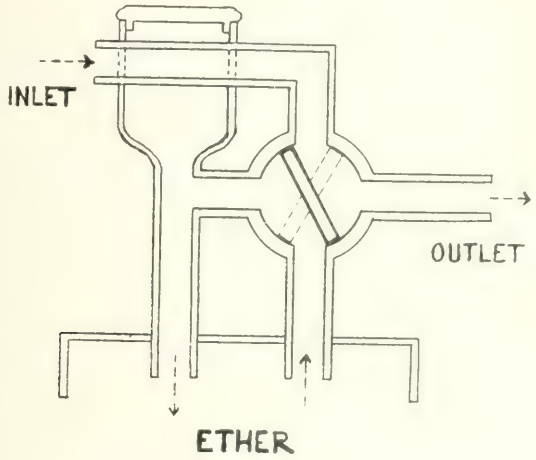

Fig. 2.

The ether valve (Fig. 2) consists of a 4-way steamcock, altered by filing away the plug so as to leave only a thin ward of metal, 1 millimeter thick at the edges. When the pointer is placed at "A" on the scale D, air from the artificial respiration apparatus passes in at the top of the valve and out through O by the most direct route. In this position both openings into the ether bottle are tightly closed, and the bottle may be inverted without danger of spilling the ether. When the pointer is placed at "E" on the scale, the air entering at the top of the valve passes into the ether bottle, over the surface of the ether, back through the valve, and out at O. By placing the pointer at intermediate positions between "A" and "E", various mixtures of air and ether vapor may be obtained. The drawing shows diagrammatically the path taken by the air in passing through the ether valve. The plug is shown in heavy lines in the position it occupies when the pointer is at "A" on the scale. The light lines show its position when the pointer is at "E".

In Fig. 1 it will be noted that the ether valve is connected to the respiration apparatus by means of a short rubber tube. This tube may be

easily removed when artificial respiration is not required and the ether bottle then used in the ordinary way. The bottle may be replenished with ether while the apparatus is in use by removing the milled cap E.

A word about the motor* may be of interest to laboratory workers. It consists of a cylindrical casing in which two pistons reciprocate under control of mechanically actuated valves. Power is transmitted by means of a rack and pinion to the shaft, which rotates through an angle of approximately 120 degrees and then reverses. There are a number of air motors on the market, and the one used in this apparatus was selected because of its excellent mechanical features and sound construction. Two types are available, operating respectively on compressed air and on suction. The first type is used in the apparatus here described. It works satisfactorily on pressures as low as 2 pounds per square inch.

The suction type of motor operates on the vacuum obtained from an ordinary water aspirator, and may be used as a source of power for rocking photographic trays, operating gas analysis apparatus, shaking Clark II ion electrodes, etc.

In this laboratory air is supplied to the machines under a pressure of 5 pounds per square inch. In an emergency the apparatus may be connected through a reduction valve to an oxygen cylinder, in which case oxygen and artificial respiration may be administered at the same time.

Eight of these machines have been in constant use in the hands of first year medical students during a period of four months, and no repairs or adjustments have yet been necessary.

*Manufactured by the Folberth Auto Specialty Co., Cleveland, Ohio.
The complete apparatus, as described, is supplied by Joseph Becker, 437 West 59th Street, New York City.

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120 (2080)

A criticism and modification of the MacLean blood sugar method.

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The titrimetric method for the determination of blood sugar proposed by Maclean[1] in 1919 has been used with success in a number of researches both abroad and in this country. Its simplicity, accuracy and the small amount of blood required recommend it to those who prefer titrimetric to colorimetric quantitative determinations. Briefly, the principle of the MacLean method is:

1. Removal of the proteins by heating oxalated blood with acidified sodium sulphate, adding colloidal iron, and filtering.

2. Boiling, under standardized conditions, an aliquot part of the filtrate with a known amount of an alkaline copper solution containing potassium iodide and potassium iodate.

3. Titration of the iodine liberated upon acidification with standard thiosulphate.

Since, under the conditions defined by MacLean, the cuprous salt reduced by the sugar is oxidized by the iodine liberated, the excess iodine titrated with thiosulphate is inversely proportional to the amount of reduction. Empirical factors can, therefore, be determined converting the volume of thiosulphate used into concentration of glucose.

[1] MacLean, H., *Biochem. J.*, 1919, xiii, 135.

It was found, however, that by MacLean's method the determinations of sugar in freshly drawn blood to which no anticoagulant had been added gave values distinctly higher than those determined on the same blood which had been oxalated, (Table I).

TABLE I.

Sugar determinations of blood to which $K_2C_2O_4$ had been added and had not been added.

Method.	Dog.	Concentration of sugar in mgs. per 100 c.c. of blood.					
		$K_2C_2O_4$ present.			$K_2C_2O_4$ not present.		
		a	b	Average	a	b	Average
Original.....	A	105	100	103	89	88	89
MacLean.....	B	91	92	92	55	55	55
Modified.....	C	90	85	88	90	85	88
MacLean.....	D	93	96	95	93	93	93

This fact led to a comparison of sugar determinations made on pure glucose solutions with and without the addition of oxalate, with and without the use of colloidal iron. The amount of oxalate required to prevent coagulation was about 2 mg. per c.c. It was found that the presence of the oxalate resulted in too low sugar values, *i.e.*, too much thiosulphate was used in the titration, only in case colloidal iron was used. Experiments in which the amount of colloidal iron was progressively increased from 0 to 3 c.c. showed that increasing amounts of thiosulphate were required for titration. Direct determinations of the iron in the filtrate indicated that the reaction involved was between the thiosulphate and the ferric salts. It seemed, therefore, that the choice of colloidal iron as a protein precipitant was an unfortunate one; although, under the carefully standardized conditions described by MacLean, it led to no inaccuracy of results, providing oxalate was not used as an anticoagulant.

By using phosphotungstic acid as our protein precipitant we were able to obtain results which agreed whether the blood was oxalated or not, and which were comparable with those obtained by the Folin method.

The procedure now adopted for the precipitation of the blood proteins is as follows:

1 c.c. of oxalated blood is added to 26 c.c. of distilled water in an Erlenmeyer flask or 50 c.c. centrifuge tube. Five minutes

are allowed for laking the blood. 2 c.c. of a 10 per cent. solution of phosphotungstic acid and 1 c.c. of 1 per cent. acetic acid are added. The flask or tube is then vigorously shaken. In the case of pigeon's blood, where precipitation is difficult, it is necessary to gently heat the mixture after the addition of phosphotungstic acid to complete the action. The protein precipitate may then be separated by filtration or preferably centrifugation. 20 c.c. of the water-clear filtrate are transferred to an Erlenmeyer flask, and the determination continued as recommended by MacLean.

The elimination of sodium sulphate from the solution results in the reduction taking place at a lower temperature. This necessitates the construction of a new table to take the place of the one published by MacLean for the conversion of c.c. of thiosulphate into mgs. of glucose per 100 c.c. of blood. These equivalents are given in Table II.

The method as now modified has been successfully applied to human, dog, ox, rabbit, guinea pig and pigeon blood.

TABLE II.

Table for the conversion of c.c. of N/100 $Na_2S_2O_3$ into mg. of glucose per c.c.

0.01N $Na_2S_2O_3$ c.c.	Glucose mg. per c.c.	0.01N $Na_2S_2O_3$ c.c.	Glucose mg. per c.c.
0.50	0.25	4.0	1.19
1.0	0.39	4.5	1.33
1.5	0.52	5.0	1.46
2.0	0.66	5.5	1.59
2.5	0.79	6.0	1.73
3.0	0.92	6.5	1.76
3.5	1.06	7.0	2.00

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1923, xx, pp. 248-252.

118 (2078)

The action of iletin (insulin) on the blood sugar of pigeons.

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The recent preparation of relatively pure and standardized extracts of the sugar reducing principle of the pancreas has been accompanied by measurements of the time and extent of the hypoglycemia resulting from its administration to the rabbit and to man.[1, 2] These earlier studies already permit the employment of such extracts with confidence in their power temporarily to reduce the blood sugar level in the higher animals. We present here the result of 120 sugar determinations made after subcutaneous injection of the extract into 35 pigeons of two species and of a third (ataxic) variety. It was usually considered unnecessary to make determinations of the sugar before injection since the birds were handled in groups and the group averages are known from extensive earlier work. These normal averages are: 185 mgms. per 100 c.c. blood (or .185 per cent.) for common pigeons[3]; 161 for ataxics; 150 for ring doves (unpublished data).

In a paper[4] accompanying this communication a pancreatic

[1] Banting, Best, Collip, Macleod and Noble, *Amer. Jour. Physiol.*, 1922, lxii, 163.

[2] Sutter and Murlin, Proc. Soc. Exp. Biol. and Med., 1922, xx, 98.

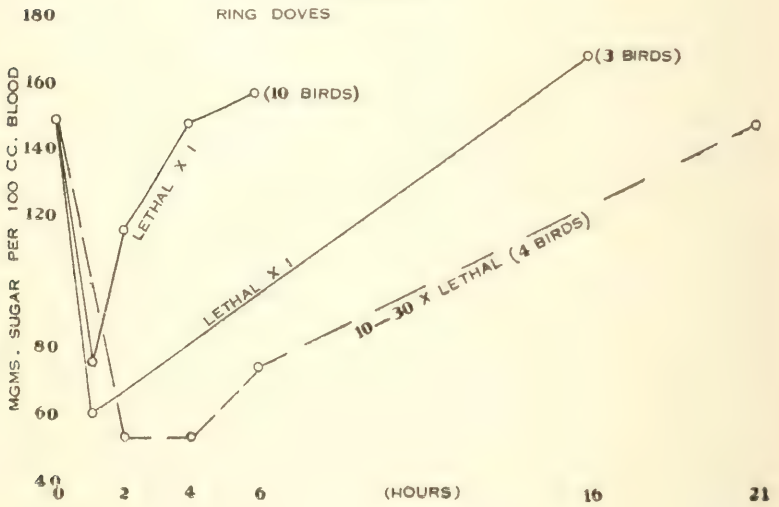
[3] Scott and Honeywell, *Amer. Jour. Physiol.*, 1921, lv, 368.

[4] Riddle, Proc. Soc. Exp. Biol. and Med., 1923, xx, [illegible].

extract of the standardized type (iletin; or insulin, Lilly) was used continuously over a prolonged period in a test of its capacity to suppress ovulations in the pigeon. Large doses were also used to test the resistance of the bird to the extract. At the close of that study the same group of birds and the same pancreatic extracts were used for the purposes of the present study. One-half of the blood samples were obtained by needle-puncture of the heart and the other half by light bleeding from the upper beak. MacLean's micro-method of sugar determination was used. Very few duplicate determinations were made. Further data concerning the extracts and dosages are given in the accompanying paper.

The curves of Figure 1 show the chief results obtained on ring-doves. In these very tame birds one can obtain samples probably unaffected by emotional glycemia resulting from handling or sampling at one-hour intervals. This is not equally possible with common pigeons and the extent of the fall of the sugar value at one hour after injection was not there measured (Figure 2). But comparison of the curves of the two groups clearly suggests that the maximum effect, of the lighter dosage at least, is reached in less than two hours in the common pigeon as is proved to be the case in the ring dove; probably this limit is attained within one hour.

FIGURE 1

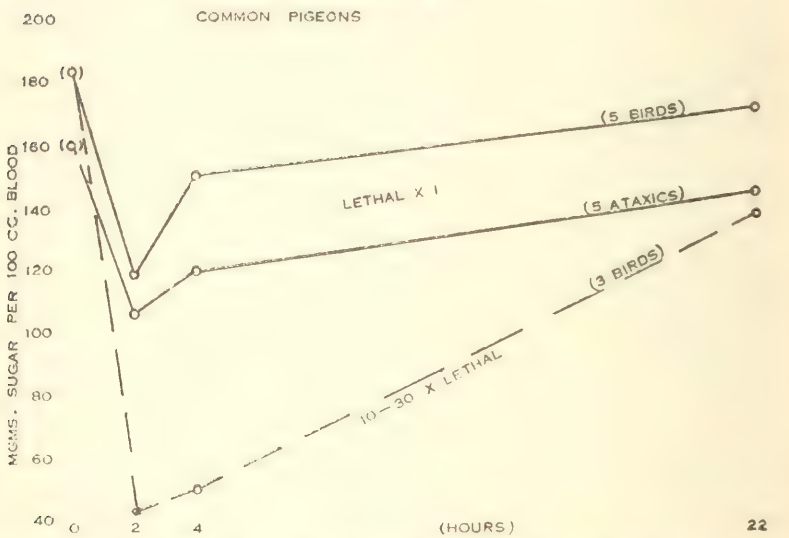


It is notable that in neither of the two groups of common pigeons given light dosage did the sugar return to a normal value at the end of four hours as it clearly did in the ring doves; nor was it at the apparent normal level after 22 hours, but our failure to obtain the normal for these particular birds before injection leaves the point uncertain. It is clear, however, that in the group of 10 ring doves (Figure 1) which at the time of sampling and during preceding weeks was being injected twice daily (9:00 A. M.; 7:00 P. M.) the blood sugar was again normal after four hours and probably above normal after six hours. Our samples from this group were drawn after the morning injection so that the last previous dosage was thus 14 hours removed. That such previous injection did not affect the sugar values as obtained under this lighter dosage is indicated by the curve itself. This point is further checked by results from the group of 3 birds given only a single injection and whose sugar tested normal or above normal at 16 hours. This group of three ring doves, each injected with one-sixth unit of a very fresh and somewhat more potent extract (Lot 2) than that given to the group of 10 ring doves, gave lower values after one hour (62 mgms.) than did eight birds (82) of the other group which were injected with Lot 1.

Four ring doves given 10 to 30 times the "lethal" dose for the rabbit (Lot 2 used) lowered the sugar value (55) at two hours much more than did the smaller (lethal X 1) dosage (97). Moreover, with the heavy dosage the sugar remained low (55) at four and at six hours. After 21 hours the curve indicates (148) a return to normal value (150); but the figures obtained from the individual birds show that neither of the seven birds for which data for this 19-21 hour period are available was even approximately normal; the figures obtained were: 25, 45, 95, 110, 230, 235, 240 mgms. per 100 c.c. of blood. The four lower values were obtained from birds given the highest dosage —30 times lethal; the three higher values were from birds given 10 (2 cases) or 20 times the lethal dose. The sugar was not determined at the one-hour period in these heavily dosed birds. The low values (20 and 30 mgms.) shown by two birds at the two-hour interval, and the wide difference between the values obtained from low and high dosage at the two-hour interval, together with the demonstrated lower level at one hour than at

two, where both were measured strongly suggest that extremely low sugar values obtain at this one-hour period under high dosage. At this particular period, however, the heavily dosed bird shows few or no wide departures from the normal in appearance or behavior. Only one of this group of birds died (after 28-34 hours); its sugar value after 21 hours was 25 mgms. Nearly all of our data from ring doves indicate that heavy insulin dosage causes a very rapid and nearly complete depletion of the blood sugar—to a point not higher than one-sixth its normal value; that this is usually followed by a some-

FIGURE 2



what slower and less complete rise; and that the short period of nearly complete absence of sugar is a period of little danger to the life of the bird.

The upper and lower curves of Figure 2 give for normal common pigeons the sugar measurements obtained from low and high dosage respectively. The much lower values from and the prolonged effect of the heavy dosage are apparent; they are also quite similar to the differences obtained with ring doves. The five ataxic pigeons figured on the middle curve were earlier known to have lower sugar values than normal pigeons. They maintained this lower level when under equivalent dosage. All these birds had a body weight of about one-third kilogram and

received therefore in the "lethal X 1" dosage about one-third unit of iletin. In the "10 to 30 X lethal" dosage they received 3 to 10.5 units of iletin. Besides having a lower body weight which results in a saving of injected material the ring doves offer several advantages over the more commonly used pigeon for laboratory studies involving the determination of blood sugar.

The blood sugar was measured in a few instances at intervals longer than 24 hours after injections. For five of these birds the sugar was not measured soon after injection and are therefore not represented on the curves noted above. Three ring doves at 34, 35 and 36 days after injection with 1 unit (6 X lethal) gave normal average values of 143, 145 and 155 for these three days; one dove given 4.5 units showed a normal value (155) at 48 hours, but less (110) at 74 hours. One common pigeon (18 X lethal) gave values of 165 after 48 hours and of only 70 after 74 hours; this bird showed only 15 mgms. sugar after two hours and survived the treatment. Another common pigeon given 3.4 units (10 X lethal) gave a normal value (185) at 32 hours and less than one-half normal (70) at 58 hours. Some of these heavily dosed birds whose sugars were low at advanced periods had probably taken no food during a considerable preceding interval..

The fact that many of the ring doves and common pigeons given heavy dosage of insulin were not permitted to take food during about 20 hours preceding dosage, and that sugar samples representing values recorded in the curves were taken as much as 22 hours after injection, raises a question as to the bearing of this circumstance upon the sugar values obtained. Concerning this point it is noted that earlier studies[5] have indicated that inanition during 48 hours is practically without effect on the normal blood sugar of the pigeon. Only 7 of the 30 birds included in the curves presented (10-30 x lethal) were in any way restricted in their feeding either before or after injection of the extracts.

[5] Honeywell, *Amer. Jour. of Physiol.*, 1921, lviii, 152.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVI. The Normal Blood Sugar of Pigeons and Its Relation to Age, Sex, Species and Certain Diseases

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Received for publication September 18, 1923

In earlier studies (1), (2) we have shown that the blood sugar of doves and pigeons markedly increases in close coincidence with each ovulation period. In the two next following studies of this series of papers (3), (4) we have demonstrated two additional factors which involve the blood sugar level on the one hand and reproduction on the other. In still another study (5) observations were made on the behavior of blood sugar values in heredity. In connection with our examination of these several relationships of blood sugar to reproduction and to inheritance it was found advisable—since absolutely homogeneous material for such studies can not be obtained—to undertake a wider survey of the relation of age, sex and certain frequently occurring diseases of pigeons to the blood sugar values shown by them; and with these data available to learn as definitely as possible the normal blood sugar concentration of a number of races, species and genera of pigeons. It is the result of this survey that is presented here.

Though it was not the main purpose of this study to throw light upon current questions concerning the mobilization and metabolism of sugar in the organism we think that some of the results bear upon these problems, since many new data are supplied for a group of species thoroughly adjusted to a high carbohydrate diet.

We may properly urge that the measurements presented are free, or practically free, from some errors which are probably included in much earlier work designed to determine the "normal" concentration of blood sugar in various animal species; for, besides care to avoid previously recognized sources of error or irregularity, three hitherto unrecognized factors which modify the blood sugar have here for the first time been consciously recognized and eliminated. These factors are: proximity to ovulation; close confinement or inactivity, and the onset of cold weather. The present results also offer the unusual opportunity of a comparison of blood sugar values obtained from seven genera belonging to a single order of animals, and of measurements extending from the embryonic period to old age within single species.

Methods and materials. Maclean's micro-method of sugar estimation has been used throughout this study. The technique employed in obtaining blood samples from the heart of adult birds has been earlier described (2), (6). Samples from embryos were obtained by decapitation and collection of the blood in pits cut into paraffin blocks. In this collection care was taken to include in the samples as little as possible of the clear serum which appears on the freshly cut surface of the musculature of the neck. From young birds aged 21 days (one week after hatching) to 3 months the samples were drawn from a wing vein. Though samples from adults were drawn by three different methods (needle-puncture of the heart; from the wing vein; and by cutting the upper beak and allowing the blood to drop into cold paraffin cups) we have observed no consistent difference of sugar content in blood obtained by these different methods. Only very small amounts of blood were taken (0.5 to 2.0 cc. unless unknown hemorrhage into pericardium occurred in cases of heart-puncture) and second or third samples were taken only at long intervals. We have found the wing vein the most practicable and accessible source of the blood. The feathers near the humeral-radial joint are drawn, the skin sponged and dried, the vein punctured with a large curved needle, and the blood allowed to collect in the pit formed by skin anterior to the joint.

The precautions taken to avoid emotional glycemia are recorded elsewhere (2). These include the very rapid collection (in less than one minute) of the sample after taking the bird in hand for this purpose. Speed was particularly sought in the case of birds unaccustomed to handling; and in three typical cases the actual time consumed in obtaining samples was 25, 28 and 42 seconds.

Until caught and removed to small cages for a period of quiet and recovery from fright, all adult birds were permitted to feed freely upon their usual mixed grain ration, since Honeywell (6) has previously shown that the blood sugar of adult pigeons is apparently but little affected by fasting during as much as 48 hours; and further because we found a 19- to 20-hour fasting period to have no measurable effect on 4 common pigeons (179 mgm.), while from 4 ring doves values higher than normal were found (168 mgm.). Since we have found no evidence of an alimentary glycemia in adult pigeons we here record the ration regularly fed to them and kept always before them: for common pigeons (and the two derived varieties): wheat, Kaffir corn, hemp, cracked maize, charcoal, grit, oyster shell, cuttle-fish bone and rock salt; for all other doves: the above ration to which millet and canary seed are added.

Though the birds usually had food in their crops, none was fed during the period of 2 to 5 hours immediately preceding the drawing of the blood samples; during this period, as noted elsewhere, the birds were trans-

ferred from their usual cages, conveniently placed in very small cages for a later blood sampling, and permitted to recover from the fright incident to being caught and transferred. For young birds being fed partially digested food by their parents an interval of fasting is required before the normal value for the adult is obtained. Table 2 gives all our data on this point. We have collected most of the blood samples between 11:00 a.m. and 6:00 p.m., but some samples were collected at earlier and slightly later hours.

The nature of the hybrids used (table 4) has been fully described elsewhere (5), and the common pigeons and ring doves used have also been sufficiently described in the preceding paper of this series (2). The "ataxic" and "scraggly" pigeons are mutational varieties derived from common pigeons. Their characteristic defects probably make them less active as adults than are the common pigeons from which they arose; the feathers of the "scragglies" do not conserve the body heat of the animal to the usual or normal extent. Neither ataxia nor scragginess is recognizable in the embryo, but embryos classified as scragglies are from matings which should yield such embryos almost exclusively. The individuals of the wild species of pigeons used (table 4) in this study were reared in captivity from imported birds. To this there are but two exceptions. The *Spilopelia suratensis* was imported, but in captivity during 3 years, and the *Leucosarcia picata* during 15 years. The exact age of all the other 415 birds used for the present study is known. For these birds a total of 709 sugar determinations is tabulated.

In most cases the birds were killed immediately after or soon after obtaining the last blood samples in order to learn whether they were healthy or diseased. Some of the birds used were, however, too valuable to kill, and this makes it impossible to classify a few of the younger groups by sex. All cases of intestinal worms (*Ascaridia maculosa*, Rudolphi) could of course be positively identified; while tuberculosis other than the earliest stages could usually be definitely identified by macroscopic observation at necropsy. Birds bearing both tuberculosis and round worms are classified in the tables as tuberculous. Excepting the 7 tuberculous common pigeons of table 1, none of the birds used was known to be diseased at the time of drawing the blood samples. Nearly all other adult birds were not only sexually mature but gave evidence of freedom from advanced disease by continued reproduction.

Three of the 24 *Turtur orientalis* taken for study (table 3) and killed for examination proved to be partial hermaphrodites—minute testes with left oviducts. One of the hybrids of table 4 had no trace of gonads or of sexual ducts; it is classified as a male (slightly tubercular). It gave a blood sugar value of 150 mgm. per 100 cc. of blood.

Practically all adult birds had been allowed to develop to maturity in a large indoor-outdoor flying space; the common pigeons in a space approximately 25 × 15 × 10 feet, the smaller ring doves in spaces approximately 15 × 5 ×7 feet. After maturity, and to the time of taking the samples, nearly all the adults birds were paired and kept in wire cages 7 × 2 × 6 feet; these were quite open to the sunshine and to fresh air except during cold weather when the sides of the cages were inclosed in glass and heat was supplied. But no data for winter conditions are given here.

All of these sugar determinations were made between March 18 and October 15. Nearly all were made between April 28 and September 15. The sugar values which we call "normal" are therefore obtained during summer. Duplicate determinations which checked to within at least 10 mgm. were obtained in most cases. In the others one to five determinations were made. In the summary of these results we have omitted those determinations which failed to check with other determinations on the same bird, or which were single determinations and fell beyond the limits of checked determinations for the race or species.

Presentation of data. *a. Tuberculosis and Ascaridia.* Seven of the 15 tuberculous birds listed in tables 1 and 3 gave abnormally high values for sugar in the blood. Two gave abnormally low values; 3 gave normal values and 3 others gave values only slightly higher than normal, if the average for the healthy birds of the group be taken as the normal. The 16 tuberculous hybrids of table 4 show a definitely lower average value than that of their 49 healthy associates (160:167 mgm. per 100 cc. of blood). Only 4 of this group had abnormally high sugar values; 3 or 4 had normal values; and 8 had abnormally low values. The details of these data therefore emphasize the tabulated averages in indicating that tuberculous birds should be excluded from others in any attempt to determine the normal blood sugar value in these species and varieties of animals. Our data show that the values obtained from tuberculous birds are usually abnormal, and that these may be either very high or very low. In our material we are unable to associate a high or low blood sugar with any particular type or degree of tuberculosis.

The effect of the presence of Ascaridia (round worms) on the blood sugar is not made wholly clear by our data. This effect seems not to be the same in the generic hybrids of table 4 as in other hybrids; nor is it the same as found for the pure species listed in tables 1 and 3. All of the four groups of Ascaridia-bearing generic hybrids of table 4 show blood sugar values higher than the normal (average of all = 176:167). Among the birds listed in table 3, however, the sugar values obtained from Ascaridia-infested birds seem definitely lower (146:164), and in table 1 they usually seem slightly lower, than the values obtained from healthy birds. But for the groups listed in table 1 this difference is slight and not really con-

TABLE 1

The blood sugar values of common pigeons (with two derived varieties, "scragglies" and ataxics) and ring doves classified as to species, age, sex and disease

KIND OF PIGEON		SEX	HEALTHY OR DISEASED	BODY WEIGHT (GRAMS)	NUMBER OF:		AMOUNT OF SUGAR IN MG. PER 100 CC. OF BLOOD)		
					Birds	Separate determinations	Minimum	Maximum	Average
Common pigeons (Columba domestica)	Adult	Males	Healthy	333	16	53	153	203	181
			Ascaridia	352	7	22	140	199	177
			Tuberculous*	337	3	6	175	245	201
		Females	Healthy	340	6	13	175	220	190
			Ascaridia	330	8	15	158	195	178
			Tuberculous*	299	4	10	178	265	202
		Average	Healthy+Asc.	337	37	103			181
	Young	Males (2.0-2.5 mo.)	Healthy (?)	324	5	10	158	210	188
			Ascaridia	373	3	6	175	185	181
		Females (1.9-2.6 mo.)	Healthy (?)	292	3	6	158	180	171
			Ascaridia	325	5	10	183	193	188
		Average	Healthy+Asc.	334	16	32			183
"Scragglies"	Adult	Males	Healthy (?)	320	5	10	128	180	150
			Ascaridia	351	6	10	140	208	176
			Tuberculous	311	1	1			110
		Females	Healthy	324	3	6	142	145	144
			Ascaridia	328	7	11	130	203	162
		Average	Healthy+Asc.	332	21	37			160
	Young	Sex (?) 2.7 mo.	Healthy (?)	294	2	4	150	160	155
Ataxics	Adult	Males	Healthy (?)	308	2	5	153	198	177
		Females	Healthy (?)	315	2	6	180	185	183
		Average		312	4	11			180
	Young	Sex (?) 2.1–2.5 mo.	Healthy (?)	320	4	7	138	210	184

TABLE 1—*Concluded*

KIND OF PIGEON		SEX	HEALTHY OR DISEASED	(BODY WEIGHT GRAMS)	NUMBER OF:		AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD)		
					Birds	Sugar determinations	Minimum	Maximum	Average
Ring doves (St. risoria and St. alba	Adult	Males	Healthy	165	11†	18	130	178	153
			Ascaridia	172	3	6	128	180	148
			Tuberculous	138	1	2			193
		Females	Healthy	163	11†	18	123	160	149
			Ascaridia	165	7	12	130	160	144
			Tuberculous	160	2	4	135	155	145
		Average	Healthy+Asc.	165	32	54			149
	Young	Sex (?) (1.9-2.2 mo.)	Healthy (?)	143	6	12	135	165	149

*Very advanced stages of tuberculosis.

†Five of these birds were not killed for examination but were probably healthy.

sistent; it would seem that the sugar values obtained from the worm-infested birds of these latter groups may be considered normal.

b. The relation of sex to blood sugar. The data for groups other than generic hybrids may be first examined. If averages obtained from healthy adult birds only be considered (tables 1 and 3), the males of the following groups seem to have slightly higher concentrations of sugar in the blood than have the females. "Scragglies," 150:144; ring doves, 153:149; mourning doves (*Zen. carolinensis*), 262:253. The females average higher in: Common pigeons, 181:190; ataxies, 177:183; *T. orientalis*, 189:192; *Stig. senegalensis*, 174:177. If the Ascaridia-bearing birds be added to the "healthy" birds—a procedure essentially justified for certain races in the preceding section—this larger number of determinations shows a similar approximation of the blood sugar in the two sexes: Scragglies, 164:157; ring doves, 152:147; common pigeons, 180:183. The three hermaphrodites (*T. orientalis*) all gave abnormal sugar values, but all were diseased. From these data we can only conclude that in these groups of birds a measurable difference of blood sugar with reference to sex in general has certainly not been found.

The healthy birds in the large groups of generic hybrids of table 4 rather consistently indicate that the males in the reciprocal crosses have a somewhat higher sugar value than the females (178:166; and 165:159). The birds bearing Ascaridia are, however, not thus consistent (183:175;

and 169:177). In these hybrids it was noted above that the Ascaridia-infested birds of all four groups show higher sugar values than do the healthy birds, though an opposite tendency is elsewhere observed in other kinds of birds thus diseased. This group of generic hybrids was of quite advanced average age (113.4 months) and it is perhaps conceivable that there was more incipient tuberculosis among the females than among the

TABLE 2

Blood sugar values of dove embryos and of young doves after various periods of fasting

KIND OF PIGEON	STAGE, OR AGE IN DAYS (AFTER EGG OF ORIGIN WAS LAID)	NUMBER OF HOURS SINCE (HATCHED) BIRDS PERMITTED TO TAKE FOOD	NUMBER OF:		BODY WEIGHT (GRAMS)	AMOUNT OF SUGAR FOUND		
			Birds	Determinations		Minimum	Maximum	Average
Common pigeons	Full-term (18 da.) embryo		4	4	10.4	146	175	157
	30–36 days	7–10	4	7	216.0	175	183	179
	35–48 days	18–19	9	14	255.0	112	190	161
	45–49 days	Not fasted	3	5	295.0	242	280	256
"Scragglies"	Full-term embryo		2	4	11.2	135	145	140
	29–43 days	9–10	3	6	212.0	128	173	155
Ataxics	39–53 days	Not fasted	3	6	285.0	175	245	208
Ring doves	Full-term (14 da.) embryo		6	6	5.1	120	153	133
	28–44 days	7–15	18	23	110.0	95	180	149
	28–42 days	16–19	21	26	94.0	115	165	143
	38–41 days	Not fasted	6	11	133.0	143	223	164
	21–25 days	Not fasted	6	9	62.0	180	213	191

males. If so, this may account for part or much of the discrepancy, since tuberculous birds of these groups have lower than normal sugar values. It is not possible to decide whether a sex difference does or does not exist in this particular division of our material.

For two additional groups of generic hybrids a much smaller amount of data is available (bottom table 3). In both of these groups the data indicate equal amounts of sugar in the blood of the two sexes (190:192 and 163:164). For most groups of birds, whether hybrid or of pure species, it can therefore be said that our data indicate an equivalence of blood sugar in the two sexes.

A special interest attaches to the figures obtained from the *Spilopelia* × *Streptopelia* cross (table 3) since the number of birds examined is fairly

large, all the birds used are full brothers and sisters, and all were killed in the first year of maturity (aged 6 to 16 months). We shall later publish data showing, within this group, a correlation between sugar concentration on the one hand and thyroid and suprarenal size on the other. The birds (males and non-ovulating females) giving lowest sugar values had larger thyroids and suprarenals; and those giving highest sugars had smaller thyroids and suprarenals. These observations plainly suggest one possible basis for the rather widely varying sugar concentration often found for different individuals of the same race or species.

c. The relation of age to blood sugar. Most of the data for blood sugar at various ages will be found in tables 1 and 2; they refer chiefly to common pigeons and to ring doves which, nevertheless, represent two different families of the order *Columbae*. Some of the young birds used were too valuable to kill and their sex and their freedom from intestinal worms is still unknown. In a previous section it has been noted, however, that neither sex nor the presence of Ascaridia appreciably modifies the blood sugar of common pigeons and ring doves. Since the averages for the adults of these groups include the birds with intestinal worms (very harmful or advanced cases of intestinal worms were not present, since only supposedly healthy and actively reproducing birds of these groups were taken for study) it is quite improbable that our failure to kill and examine these young birds appreciably affects the values listed for them.

Combining the stages given in tables 1 and 2 we find as follows for common pigeons: embryos, 157; 1.1 months, 179; 2.3 months, 183; adult, 181. For "scragglies": embryos, 140; 1.2 months, 155; 2.7 months, 155; adult, 160. For ring doves: embryos, 133, 1.2 months, 149; 2.1 months, 149; adult, 149. These data indicate that the normal amount of sugar in the blood of the adult rather closely approximates to that present at the end of embryonic life and at intervening periods. It is further indicated that the amount of sugar present in the blood of the three kinds of embryos is somewhat less than in the adults of the corresponding species, but that the differences found in the blood of the embryos is as characteristic of the species as is that of the adult. These points can be seen to advantage in figure 1.

Two points in the data just given require further consideration. The first of these is the probability that the blood samples obtained from the decapitated embryos were slightly contaminated with the colorless serum which slowly filters from the freshly cut tissues of the neck. The drops of blood are of course also in momentary contact with at least a part of this cut surface and doubtless carry away traces of it. We have been unable to collect a sufficient quantity of this serum to make a test of its reducing capacity. But it probably has a lower sugar concentration than has the blood, and we suspect that its admixture with the blood sample is the source

of the relatively slight difference observed in the blood of embryo and adult. The second point concerns the amount or degree of fasting necessary in order to obtain a "normal" blood sugar from young birds still being fed partially digested food by the parents. Or, perhaps the real question is, what is the "normal" in such young birds? For the purposes of the list of sugar values given above, and for the construction of figure 1, we have chosen a value (for birds about 1.2 months old) on the basis of reasons which may next be given.

The data of table 2 demonstrate that while being fed by their parents, and during those hours of the day when their "crops" are filled with food, young pigeons show a definite hyperglycemia. Much higher values are then regularly found than is found for the embryo, the adult, or for these same young late at night or before feeding in early morning. After 7 to 10

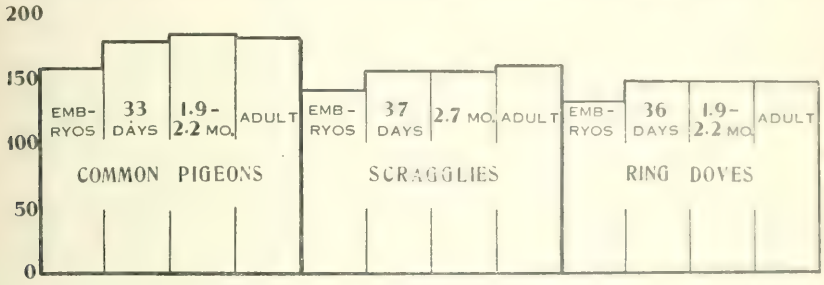

Fig.1. Polygons showing the amount of blood sugar for each of three kinds of pigeons at four different periods of life. The ordinates represent milligrams sugar per 100 cc. of blood.

hours of fasting the young of common pigeons show a sugar value, 179, which is practically equal to that of the adult, 181. After 18 to 19 hours of fasting the sugar value in such young is plainly lower, 161, than that of the normal adult. A fasting period of 18 to 19 hours is longer than the normal interval between the last evening-feeding and the first morning-feeding period in pigeons, i.e., it is longer than any naturally occurring fasting period. It can certainly be said that a fasting period of 7 to 15 hours actually occurs daily in the life of the bird. Similarly, very young "scraggly" offspring showed a sugar value of 155 after 9 to 10 hours fasting as compared with a normal adult value of 160. And finally, the young ring doves fasted during 7 to 15 hours gave a wholly normal sugar value of 149. All of these groups show that a longer period of fasting is accompanied by sugar concentrations lower than normal, and if not fasted at all these groups of young—still being fed partially digested food by parents—all show a sugar concentration higher than is found at any other time in their life history.

TABLE 3

The blood sugar values of various doves and pigeons classified as to species, sex and disease

KIND OF PIGEON	SEX	HEALTHY OR DISEASED	BODY WEIGHT	NUMBER OF:		AMOUNT OF SUGAR (MGM. PER 100 CC. OF BLOOD)		
				Birds	Sugar determinations	Minimum	Maximum	Average
Turtur orientalis	Males	Healthy	226	13	26	160	205	189
		Tuberculous	221	2	2	190	250	220
	Females	Healthy	223	5	9	175	210	192
		Tuberculous	231	1	1			225
	Unknown	Healthy (?)	230	4	8	180	202	191
	Hermaphrodites	Ascaris	218	1	2			155
		Tuberculous	239	2	4	200	205	203
	Average	Healthy	226	22	43			190
Stigmatopelia senegalensis	Males	Healthy (?)	96	3	6	162	195	174
	Females	Healthy (?)	96	2	3	170	185	177
	Unknown	Healthy (?)	90	4	6	150	175	158
	Average	Healthy (?)	94	9	15			168
Zenaidura carolinesis	Males	Healthy (?)	124	1	3	255	270	262
	Females	Healthy	120	1	2	250	255	253
Spil. chinensis	Females	Healthy (?)	170	1	3	160	180	173
Spil. suratensis	Male	Healthy (?)	180	1	3	165	175	170
Leuc. picata	Male	Healthy (?)	420	1	5	180	200	190
♂ Spil. suratensis × ♀ Streptop. hyb.	Males	Healthy	143	9	15	130	185	163
		Ascaridia	149	5	10	125	175	142
		Tuberculous	129	5	8	130	200	154
	Females	Healthy	141	11	23	135	208	164
		Ascaridia	150	5	12	135	172	149
		Tuberculous		2	4	150	183	166
	Average	Healthy	142	20	38			164
Zenaida-Zenaidura hybs.	Males	Healthy (?)	97	2	3	180	200	190
	Females	Healthy (?)	95	2	3	185	200	192

The above data show that the normal concentration of blood sugar is obtained in young common pigeons and in the seragglies after 7 to 10 hours fasting; and that in ring doves this value is obtained after 7 to 15 hours. The fact that hyperglycemia regularly occurs in these very young pigeons during the several hours of each day of the feeding period suggests either that the efficiency of the glycogenic functions is not yet fully established at this stage; or that the absorptive powers of the young bird's intestine—crammed with food already partially digested—are quite out of proportion to the absorptive powers possessed by the bird a month later (see table 1).

The associated fact—that so short a period of fasting as 16 to 19 hours results in a hypoglycemia in these young birds—is equally interesting, since Honeywell (6) has shown that a fasting period of even 48 hours has little effect on the blood sugar of adult common pigeons. Also our more recent tests show that no hypoglycemia results, either in common pigeons or in the ring dove adults, from fasting 19 to 20 hours. This very different result in very young and in adult pigeons is probably associated with the extraordinary rate of growth which is prevalent at this stage in the life of the young birds; but it is clear that an alimentary glycemia is present in the young birds being fed partially digested food, and that in this same stage the blood sugar of the young pigeon is affected by short periods of fasting. For other stages of life there is no evidence that an alimentary glycemia occurs in the pigeon.

On the question of blood sugar concentration in birds of very old age we have the following data: The five youngest (11.1 months) healthy *T. orientalis* (table 3) gave an average of 183; the five oldest (56 months), 188. The 10 youngest (adult) common pigeons (7.5 months) average 177; the 10 oldest (20 months) average 181. The 5 youngest adult "seragglies" (6.6 months) average 164; the five oldest (16.2 months) average 161. The 10 youngest (adult) ring doves (8.4 months) average 151; the 10 oldest (47 months) average 149. The 21 healthy male and female generic hybrids of table 4 which were aged 75 to 100 months gave an average sugar value of 168; while 11 similar hybrids aged 130 to 183 months averaged 171. From these data, and from an examination of the details on which the averages are based as well as from smaller amounts of data obtained from the smaller groups of birds, we find it wholly improbable that very old age in pigeons is normally accompanied by any change in the concentration of sugar in the blood.

It can therefore be said that the data obtained for common pigeons and ring doves at several ages, extending from the unhatched embryo to old age, lead to the conclusion that the normal concentration of reducing substances in the blood normally remains at the same level throughout the whole life cycle. The amount found for the embryos is somewhat less than

that found for all later stages; but the difficulty of obtaining blood samples of embryos uncontaminated with a serum pressed from the cut tissue is probably the explanation of this deficiency.

d. The normal blood sugar of various species and races. Sugar determinations have been made on nine species, two derived races and on three

TABLE 4

Blood sugar values (non-ovulation stages) of a group of generic hybrids classified as to direction of cross, sex, health and disease; average weight of body, suprarenals and thyroids given for all classes

KIND OF HYBRID	SEX	HEALTHY OR DISEASED	NUMBER OF SUGAR DETERMINATIONS	NUMBER OF BIRDS	BLOOD SUGAR (MGM.)	AVERAGE WEIGHT OF		
						Body (grams)	Suprarenals (mgm.)	Thyroids (mgm.)
♂ T. orientalis × ♀ Strep. alba	Males	Healthy	25	14	178	194	16.2	26.7
		Ascaridia	6	3	183	210	16.3	24.5
		Tuberculous	6	3	159	208	19.1	25.0
	Females	Healthy	20	11	166	195	12.8	20.3
		Ascaridia	2	1	175	204	12.3	26.0
		Tuberculous	2	1	185	170	22.6	21.6
♂ Strep. alba × ♀ T. orientalis	Males	Healthy	20	11	165	204	14.6	24.2
		Ascaridia	8	4	169	192	12.8	24.2
		Tuberculous	10	6	154	208	21.2	26.4
	Females	Healthy	21	13	159	189	12.6	24.9
		Ascaridia	12	6	177	180	13.4	22.1
		Tuberculous	12	6	143	181	17.3	47.2
Totals or means	Male	Healthy	45	25	171	199	15.4	25.5
	Female	Healthy	41	24	163	192	12.7	22.6
	♂ and ♀	Healthy	86	49	167	196	14.1	24.0
		Ascaridia	28	14	176	197	13.7	24.2
		Tuberculous	30	16	160	192	20.1	30.1

groups of generic hybrids. For three or four of these groups the number of observations made and of birds used is too small to afford very definite information. Within the nine species seven genera are represented, and these genera belong to two families and four different sub-families of Columbae. The data of tables 1 to 3 make it fairly clear that all except two of the species examined from these genera are characterized by distinct and different concentrations of reducing substances in their blood. A list of

these genera with the adult sugar values found for them, together with the number of birds and of observations placed in parentheses, is given below.

Streptopelia	wt. = 165 grams (32–54);	sugar = 149 mgm.
Stigmatopelia	wt. = 94 grams (9–15);	sugar = 169 mgm.
Spilopelia	wt. = 175 grams (2–6);	sugar = 172 mgm.
Columba	wt. = 337 grams (37–103);	sugar = 181 mgm.
Turtur	wt. = 226 grams (22–43);	sugar = 190 mgm.
Leucosarcia	wt. = 420 grams (1–5);	sugar = 190 mgm.
Zenaidura	wt. = 122 grams (2–5);	sugar = 258 mgm.

The weights given in this list are of course for the species and individuals actually used in the study. It is quite clear that there is no relation between the size of the species studied and the percentage of sugar in the blood.

Discussion and interpretation. Reference should here be made to previous measurements of blood sugar in birds. The nature and extent of this work, together with many points facilitating its consideration, can be most conveniently presented in the form of table 5. The tabulated data make it clear that but little work has been done with the purpose of learning the normal concentration of sugar in the blood of birds. Only four distinct kinds of birds have hitherto been studied and among these there is probably not a single pure or natural species represented. The "fowls" studied by the different observers may have been very different races—probably more different than our common pigeons and the "scragglies" derived from them; and these two groups we have shown to be characterized by two different blood sugar levels. The same would apply to the various studies on "ducks" and "geese." Common pigeons too are mongrels, not a species, and nearly 200 such races exist. The common pigeons used by us are a mixture of "homer" and "carrier," and in some cases the "Jacobin" is also represented in the cross. These birds therefore correspond fairly closely in their origin and composition to the "pigeons" usually available as laboratory animals in this country. In this connection it should be recalled that we have elsewhere (5) shown that blood sugar levels are inherited in crosses.

Table 5 also shows that quite different values have been obtained for the fowl by the various investigators; and the same is also true in the case of the duck. We are tempted to assume that much of these differences is also assignable to different degrees of emotional glycemia and to different periods or degrees of confinement prior to the taking of the samples. In the work of Scott and Honeywell (13) hyperglycemia due to fright was probably successfully avoided; and a comparison of the values obtained for the common pigeon by them with those obtained by us indicates that their birds were not affected by too close confinement. In practically none of the other studies listed is it possible for the reader to learn that either of these two important factors was excluded.

It is further notable that Flemming (12) obtained values (75 mgm.) for ducks, and Marañon and Rosique values for the dove (80 mgm.) which are wholly at variance with all other observations on birds and which are quite similar to values found for mammals. Flemming's several determinations are consistently low; but he makes no note of the wide discrepancy between his own and earlier findings. Unfortunately only the abstract of the work of Marañon and Rosique has been available to us. Unless the low values found in the two above-mentioned studies were the

TABLE 5

A summary of earlier measurements of the blood sugar in birds

BIRD	NUMBER	AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC.)		METHOD USED	OBSERVER
		Range	Average		
Fowl	8	188–250	209	Abeles	Saito and Katayama (7)
Fowl (adult)	4	175–190	183	Bierry-Portier	Giaja (8)
Fowl (2 mo.)	4	200–240	220	Bierry-Portier	Giaja (8)
Fowl	?	? ?	140	?	Bernard (cited from Bang)
Fowl	?	? ?	230	Bierry-Portier	Bierry and Fandard (9)
Duck	7	120–200	160	Abeles	Weintraud (10)
Duck	14	120–180	146	Abeles	Kausch (11)
Duck (fasting)	5	140–160	150	Abeles	Kausch (11)
Duck (sick)	5	130–150	140	Abeles	Kausch (11)
Duck	12	50–98	75	Pflüger-Allihn	Flemming (12)
Geese	6	120–160	138	Abeles	Kausch
Common pigeon	22	160–239	185	Maclean	Scott and Honeywell (13)
Dove ?	?	? ?	80	Bang	Marañon and Rosique (14)

result of prolonged and extreme confinement of the animals used we are wholly unable to interpret them.

Only Giaja (8) has compared the blood sugar of the young and adult of the same race and he made but four observations on each group. He concluded, however, that young fowls—aged about 2 months—have clearly higher sugar values than fowls aged at least one year. The value obtained by him for the adults was, however, considerably less than that obtained by Saito and Katayama (7). The high values obtained by us for one stage of young pigeons we have interpreted as a hyperglycemia resulting from their being fed partially digested food by the parents: there being either failure of full development of the glycogenic function, or an excessively rapid absorptive rate from the intestine, at this stage of growth. This type of feeding does not exist in the fowl: if therefore the young of the fowl should later be proved to have a higher sugar value than that of the adult

it would be made clear that our interpretation of this point is inadequate or wrong.

Kausch (11) states that fasting up to 72 hours does not measurably lower the blood sugar of ducks. Here, however, one would wish to know whether or to what extent emotional glycemia entered into the values found. Honeywell (6) was unable definitely to decide whether 48 hours of inanition had an effect on the blood sugar of 35 common pigeons; the birds used in that study were obtained from dealers and the values found (166 before and 165 after) were lower—both before and after the inanition period—than is now recognized as normal for common pigeons. As earlier noted, this point was further examined by us in tests of the effect of a 20-hour period of fasting and no decrease was found. From these several results, and limiting our statement to the kind of food and method of feeding used by us, we can state as follows: It seems probable that in the pigeon—an animal using a high carbohydrate diet but unlike the rabbit in feeding only by day—the only notable alimentary glycemia which occurs is that during feeding by the parents in the short period of immaturity. This point probably involves a notable difference in one or another aspect of the carbohydrate metabolism of mammals on the one hand and of birds like the pigeon on the other. The alleged greater difficulty of obtaining a pancreatic glycuresis and glycemia in birds may be related to this same difference.

The measurements made on the blood sugar of several wild species of birds, as carried out in the present study with the elimination of many factors known to modify such results, make it quite certain that in at least one large order of birds a very high concentration of reducing substances is normally present in the blood. The different genera and species of Columbae also further show relatively wide differences in normal concentration of sugar in the blood.

SUMMARY

Measurements of the normal concentration of the reducing substances of the blood were made on 415 birds belonging to nine species, two derived races, and three groups of generic hybrids. These species include seven genera and two families of *Columbae*. Most of these species and genera are found to have different and characteristic concentrations of blood sugar. The species examined vary between 149 and 258 mgm. sugar per 100 cc. of blood.

The factors previously known to modify the normal sugar level have been considered, minimized or eliminated from the results; and in addition the data obtained are free from errors arising from three hitherto unrecognized factors—proximity to ovulation, prolonged inactivity or close

confinement, and the onset of cold weather. The nature and condition of the animals used are fully described.

Tuberculous birds usually have abnormal concentrations of sugar in the blood; this concentration may be either high or low. The presence of round worms (*Ascaridia maculosa*) in the intestines does not seem to affect the blood sugar values of all species and hybrids in a similar manner. In reproducing common pigeons and in ring doves the concentration of sugar was unaffected.

Males and non-ovulating females do not show consistent or unquestionable differences in blood sugar.

There is no relation of the size of the races and species studied to the normal concentration of the sugar of the blood.

The blood sugar has been measured in the embryo, in two juvenile stages, in adult stages, and in old age. The values obtained for these several ages are notably similar in any single species or race. The values actually obtained for embryos are slightly lower than for adults, but the apparent difference is probably not real.

In that part of the juvenile period during which the young is being fed partially digested food by the parents, the hours of active food absorption are characterized by values higher than those of the normal adult. In these cases the normal adult values are obtained only after 7 to 15 hours of fasting. It is only during this part of the growth period that an alimentary hyperglycemia has been found in pigeons.

The normally high concentration of the blood sugar of birds, the relative freedom of pigeons from alimentary hyperglycemia (despite the high carbohydrate diet of these species), and the stability of their blood sugar concentration under shorter periods of fasting, all suggest that they differ from mammals in one or another aspect of their carbohydrate metabolism.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVII. Blood Sugar and Ovulation under Inactivity or Close Confinement

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It has long been recognized in the practical breeding of pigeons and of poultry that ovulation ceases soon after reproducing birds are confined in spaces so small as to prevent or greatly restrict the normal movements of the animal. This experience has been so general that data on this point need not be submitted here. The physiological question involved is, what changes in the organism are responsible for, or are associated with, the suppression of ovulation by this confinement and inactivity?

Early in our studies on the blood sugar of pigeons it was thought that birds kept in small cages were probably unsuitable for use in determining the normal blood sugar of a race or species. Lusk [1] had observed on the dog that "prolonged confinement in a cage will, after a time, greatly reduce the basal metabolism though there be no loss in weight. Recovery from this condition is achieved through exercise." This observation suggested to us that metabolic disturbances are involved in the loss of reproductive power by birds kept under these conditions. It therefore seemed advisable to test the effect of such confinement on the concentration of blood sugar. Making this test we should learn: First, whether the suppression of ovulation from these sources is accompanied by changes in carbohydrate metabolism as reflected by the sugar concentration in the blood. Second, whether such inactivity and confinement is a factor modifying the normal blood sugar.

Experimental. Blood samples were obtained by needle-puncture of the heart under precautions described in our preceding studies, and the sugar determined by Maclean's micro-method. In this study two groups of birds were used. Series I was composed of 18 common pigeons which had grown to maturity in a large (25 × 15 × 10 feet) outdoor-indoor flying space. For two months prior to the drawing of the control blood samples the 18 birds had occupied an "outdoor" space 15 × 5 × 7 feet. After obtaining duplicate sugar determinations for these 18 birds on June 13 to 16, they were divided into lots A and B. Lot A, composed of 8 birds

(taken from the head of the list of first samples drawn) which had given an average sugar value of 181 mgm. per 100 cc. of blood, was used as a first control and therefore left in their former quarters. The remaining 10 birds, lot B, were removed to a very small cage ($3\frac{1}{2} \times 2 \times 2$ feet). This cage was placed in a well-ventilated but only partially lighted basement of the laboratory-annex. The poorer lighting was sought in order to insure less dissension and less activity. This lighting was, however, fully adequate to permit the birds to feed and drink freely. An unwished for but unavoidable factor associated with these basement quarters was a somewhat lower temperature (5° to 15°F.) during the hours of sunshine.

TABLE 1

Summary on the effects of inactivity or confinement in small space on the amount of blood sugar in pigeons

SERIES	GROUP	NUMBER OF BIRDS	MILLIGRAMS OF SUGAR PER 100 CC. OF BLOOD			
			Control (normal activity)	Control		After 3–5 weeks confinement and inactivity
				Cont'd.	After inactivity	
I	A	8	181	181		132
	B	10	175		(173)	119
II	A	3	177	180		
	B	4	178			127
Average			178	181	(173)	125

Before removal to their very limited quarters lot B had an average sugar concentration of 175 mgm. On July 5—after twenty days of close confinement—lot B gave an average blood sugar of 119. Lot A, which had remained in their former quarters again averaged 181. At this time (July 5) the position of the two lots was reversed by the return of lot B to their former quarters, and the subjection of lot A to the small basement cage. Five weeks later (August 12 to 14) lot B, now used as a control, gave an average sugar value of 173; and lot A, now the test animals, gave an average of 132. These data are summarized in table 1.

Series II, composed of 7 additional birds, was begun on July 5. The 3 birds of lot A of this series, then giving an initial sugar value of 177, were left in the larger flying pen. The four birds of lot B, which had just shown a normal average value of 178, were placed (with birds of series I) in the small pen. After 5 weeks lot A had an average sugar concentration of 180; lot B, an average of 127.

At the beginning of the experiment the birds of lot A were aged 9.0 months; those of lot B averaged 9.6 months. At this period the average body weight of lot A was 315 grams; that of B, 324 grams. Lot B neither

lost nor gained weight during the period of close confinement (324 grams at close) though the control birds slightly increased their weight to 330 grams during this same period. During its five weeks of close confinement lot A showed a slight loss in weight—to 310 grams. But the two birds of this group which gained weight during this period showed the same change in sugar values as did those which lost weight. In general, in these experiments the sugar values were about equally depressed in birds which gained weight and in those which lost weight during close confinement.

Other data obtained by us clearly indicate that the somewhat lower temperature of the basement room where the birds were closely confined (June to August) can not be the factor responsible for the fall in sugar concentration. These data (2) showed that the temperature of September—though bringing a greater lowering of temperature from that of June than that involved in the change to basement quarters—is without measurable effect on the blood sugar of common pigeons.

Discussion. Three to five weeks of largely suppressed activity associated with confinement in very close quarters was accompanied by a reduction in the blood sugar of 22 birds from an average of 178 to 125—a reduction from the normal of 29.8 per cent. The reversal of these conditions in lots A and B of series I was promptly accompanied by a reversal of the values found; and return to normal conditions was followed by a return to the normal and previously observed concentration of sugar in the blood. Three additional birds showed an initial normal average of 177 and, as a further control, fully maintained a normal average (180) during the five weeks of inactivity in which their associates (series II) showed a fall in values from 178 to 127.

It is therefore clear that the blood sugar concentrations found in these animals are greatly influenced by confinement; and that, if really normal sugar concentrations are sought in these animals, this factor must be eliminated. There is every probability that these findings apply also to the other laboratory animals commonly used in studies on the blood sugar. We believe that failure to eliminate this factor is in part responsible for the different sugar values reported for the same race of animal by various observers.

In our experiments not a single egg was ovulated under confinement though they were being produced in the period before confinement. This conforms to general breeding experience with birds. It thus becomes clear that in birds closely confined *one* condition accompanying the suppression of their ovulation is a great and rapid reduction of sugar concentration in the blood. This point gains added significance through the similar additional observations made by us: First, that the sugar of the blood normally and regularly *increases* at each ovulation period in the pigeon (3), (4). Second, that a dosage of insulin which twice daily lowers the

blood sugar during 1 to 4 hours to only 60 to 80 mgm. is sufficient to prevent most ovulations in pigeons (5). Third, in the next following paper of this series (2) it has been shown that the onset of cold weather—well known to decrease or suppress ovulation in many birds—also causes fluctuations, certainly involving a temporary decrease, in the sugar of the blood. It is thus made clear that these several conditions which tend to lower the blood sugar concentration tend also to suppress ovulation.

CONCLUSIONS

Close confinement with enforced inactivity produces within three to five weeks a decrease of nearly 30 per cent in the normal blood sugar of pigeons. This fact is doubtless definitely related to Lusk's observation that these same conditions greatly reduce the basal metabolism of the dog.

The hypoglycemia incident to the laboratory or other confinement of animals or of man is probably a widespread source of error or of difference in past and current "normal" blood sugar measurements.

These conditions—close confinement and inactivity—result in the suppression of ovulation in pigeons even though the confined animal makes slight gains in weight.

Several facts suggest that ovulation in birds is normally associated with the capacity of the organism temporarily to increase the blood sugar to a value higher than the normal concentration, and that conditions adverse to this capacity tend to suppress ovulation.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XVIII. Effects of the Onset of Cold Weather on Blood Sugar and Ovulation Rate in Pigeons

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It has been found that the blood sugar is temporarily decreased in pigeons which are unprotected from the onset of cold weather in late autumn. At the same time and under these same conditions the rate of ovulation is also decreased in these animals. During October, 1922, it was possible to identify the specific date of the first severe autumn weather with a pronounced decrease in both the sugar concentration and the ovulation rate in several kinds of pigeons.

These results make it clear that "normal" concentrations of blood sugar may not be obtained from pigeons similarly and recently subjected to cold; and the same will probably also apply to other birds and to mammals. They further show that a reduced ovulation rate is associated in this instance, as in others previously described by us, with a reduction in the blood sugar. It is also obvious that the diminution of the sugar of the blood under these conditions is an item of interest in connection with the greater heat production, as well as possible changes in basal metabolism, which doubtless occurs in coincidence with it.

Consideration of data. *Effects of cold on blood sugar.* The beginning of this study was not planned as a study of the effects of cold on the blood sugar; these effects were encountered in the pursuit of information on normal sugar values. A summary of the sugar concentrations obtained from various kinds of pigeons at various periods with reference to the onset of cold weather is given in table 1. It will be seen that three of the four kinds of adult pigeons maintained their normal (summer) blood sugar concentrations to October 15. But the birds of these three adult groups, together with corresponding groups of young birds which were examined between October 20 to 29, all gave lower than normal sugar values (the adult ring dove value—148—seems insignificantly reduced). The fourth group—the "scraggly" common pigeons—gave reduced sugar concentrations as early as the end of September. The special case of this imperfectly feathered variety will be considered later.

Our explanation of this apparent reduction of sugar values may next be given. On October 19 and 20 the birds were exposed to a fall of the external temperature to 30° and 33°F. This involved a sudden temperature fall of about 10°F. below the minimum of any preceding day of the autumn; and on both of these days the low temperatures were accompanied by winds of relatively high velocity (mean, 19.3 and 21.0 miles per hour) which added much to the cooling power of the air.

The adult ring doves listed in table 1 do not show a significant effect of the cold weather when considered as a single group. Probably this is because five of the nine birds examined were chosen from the one of our

TABLE 1

Summary on variations of blood sugar during onset of cold weather

KIND OF PIGEON	AMOUNT OF BLOOD SUGAR (MGM. PER 100 CC.)									
	April 28 to September 15		September 21 to October 8		October 13 to 15		October 20 to 29		November 18 to 20	
	No.	Mgm.	No.	Mgm.	No.	Mgm.	No.	Mgm.	No.	Mgm.
Common pigeons	37	181	6	182	6	190	7	175*	3	180
Common pigeons (young)	16	183					8	143	8	185
Scragglies	21	160	16	148	8	140	2	150	3	149
Scragglies (young)	2	155			2	134				
Ataxics	4	180	1	183	1	188	4	150	1	175
Ataxics (young)	4	184	1	155	1	160	2	159		
Ring doves	26	149	6	149	3	149	9	148		
Ring doves (young)	6	149					4	121		

* Four of the seven tests were on "ovulation" stages and should have shown higher than normal sugar concentrations.

five buildings best protected from cold and wind. The blood samples from these five birds were drawn on October 20 and 21. This was before any effects of cold weather were understood, but it was nevertheless thought advisable to use birds least exposed to cold since "normal" sugars were sought and cold weather was an untested factor. The five birds chosen from protected quarters showed slightly more (153 mgm.) than the normal sugar value (149 mgm.); and the four adult ring doves which were taken from more exposed locations showed slightly less (142 mgm.) than normal sugar. The young ring doves all clearly showed lower than normal values (average, 121 mgm.). In reality therefore it may be said that the ring doves, like the common pigeons and ataxics, showed decreased amounts of blood sugar after severe and prolonged exposure to cold. The data

suggest that the effects of cold appeared earlier and were more severe in the young birds than in the adults of the various groups.

The summaries of table 1 give data for kinds or groups of birds at various dates, but measurements made on the same individual at various dates

TABLE 2

Blood sugar tests on individual birds before and after onset of cold weather

KIND	NUMBER OF BIRD	TIME OF TAKING EARLIER SAMPLES	AMOUNT OF BLOOD SUGAR (MGM. PER 100 C.C.)		
			From earlier samples	October 20 to 29	November 18 to 20
Adult common pigeons	1	Sept. 22–24	195	180*	
	2	Sept. 24	195	175*	
	3	Oct. 7–8	183	170*	
	4	Oct. 14–15	193	178*	193
	5	Oct. 13–14	198*		168*
	6	Oct. 7–8	183	175	
	7			183	180
	Average		*191*	*177**	*180*
Young common pigeons	8			128	183
	9			128	175
	10			125	185
	11			155	188
	12			130	185
	13			150	183
	14			133	193
	15			153	190
	Average			*138*	*185*
Adult ataxic	1	Oct. 6–7	*185*		*175*
Adult scragglies	1	Sept. 23	175	145	
	2	Sept. 23	145	155	
	3	Oct. 7–14	128		153
	4	Oct. 7–14	138		153
	Average		*147*	*150*	*153*

* Ovulation stages and the blood sugar should have been higher than normal.

are not separately presented there. The data for individuals examined after the onset of cold weather, and also at an earlier or a still later period, are listed in table 2. Inspection of these data will show that they fully support the conclusions drawn from the combined data of table 1.

The "scraggly" pigeons are a mutational variety derived from common pigeons. In these birds all of the epidermal system is affected and none of the feathers are normally formed; the birds cannot fly because the wing

feathers offer too little resistance to the air; also, the loose and deficient feathering is obviously incapable of a normal conservation of body heat, so that birds of this type can survive the winter only when kept in heated buildings. It is significant therefore that the weather of the early autumn period—even as early as the end of September—was sufficient to cause a reduction in the blood sugar of these birds. In this case it is also notable that the reduced sugar concentrations persisted (table 1) during the period of nearly two months covered by the measurements. The earlier decrease of the blood sugar and the longer continuance of this decrease in these scragglies, together with the results from the five ring doves from protected quarters, indicate that the observed decrease is not a purely seasonal fluctuation or annual rhythm unrelated to cold.

It should be pointed out that after October 30 all of the birds were given some protection against cold, and were completely protected against wind, by the replacement of the glass sides of the buildings in which they were confined. It will be noted that three weeks after providing this partial protection from cold the blood sugars of common pigeons and of ataxics had again become apparently normal. The reduced sugar values persisted, however, in the scragglies. No data were obtained for ring doves at this period. We do not feel able to speak confidently concerning the time and conditions necessary for a recovery of normal sugar values in any birds except the young common pigeons. In this case it is clear (table 2) that the normal sugar value was restored from a very low level within a period of three or four weeks. Further definite data concerning the temperatures of the period covered by this study are given in the following section and in figure 1.

Effects of cold on the rate of ovulation. We have attempted to show the fluctuations of ovulation rate in relation to the external temperatures by means of curves in figure 1. The temperature curve is based on the records of the Weather Bureau in New York City (32 miles distant). Those temperatures do not very accurately describe the local temperatures at Cold Spring Harbor where the temperatures were usually lower during this period. All of the very low local temperatures were recorded during this period, and the four arrow-heads on the curve represent these lowest local daily temperatures as registered prior to November 25. These lowest minimum daily temperatures occurred on October 19, November 10, 14 and 24. Our lack of a complete record of local temperatures seems to require the use of the New York City temperatures for purposes of the curve.

The number of eggs laid may here be taken as a sufficiently close measure of the number of ovulations. If the number of eggs laid during 5-day periods, from October 1 to November 24, be plotted in the form of curves, we obtain results indicated in the figure. The two most numerous groups

or kinds of pigeons in our collection are here figured separately. The rate of ovulation in the ring doves—from which larger numbers and more reliable data were obtained—promptly decreased after the two periods of notably low temperatures. The common pigeons show some or much decline in ovulation rate slightly preceding these periods of lowest temperatures, but minimal rates follow both periods of lowest temperatures. Both ring doves and common pigeons show at least partial recovery coincident with the general rise in the temperature curve (October 30 to November 9)—a period also coincident with their partial protection from cold as a result of the enclosure of their quarters.

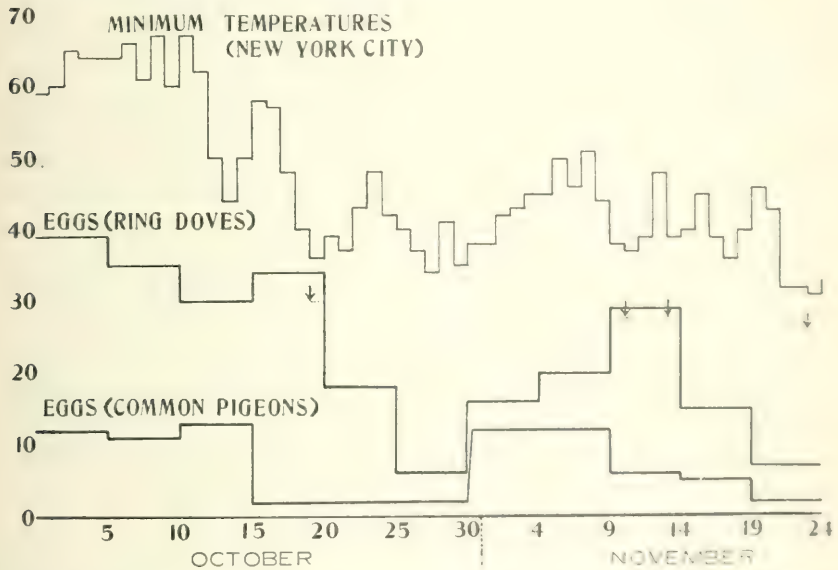

Fig. 1. Showing curves for the ovulation rate of common pigeons and ring doves during the approach of cold weather—October and November, 1922. Days of the months on base line. The ordinates represent the number of eggs produced (ovulations) in 5-day periods, and also temperature (degrees F.). Upper curve, minimum temperatures of New York City. The arrow-heads show local minimum temperatures.

By still other data it can be shown that the season of increasing cold—October 1 to January 1—is characterized by a reduced rate of ovulation in pigeons. The temperature curve of figure 1 indicates a general decline of temperature during October and November, 1922. The records for December are not figured but they show a similar decline. In table 3 we have summarized data on the rate of egg production by 15 individual ring doves for a period which includes all of the year 1922, and therefore the period covered by figure 1, together with the succeeding winter period—

January 1 to April 1. The 15 birds selected for tabulation are those showing highest egg production among a group of 40 birds given opportunity to produce the maximum number of eggs during this period.

The tabulated data clearly show that the period of falling external temperature—October to January—is the period of lowest egg production (107); and that the highest rate (280) is from April 1 to July 1. During this latter period the temperature is already fairly high and is continually rising. That the decline in ovulation rate is not the result of sickness, with permanent suppression of egg production toward the end of the year, is shown by the rise in ovulation rate during the next following 3-month period. The details of the data further show that the period from October 1 to January 1 is also a period during which a high percentage of clutches of "single" instead of "paired" yolks are ovulated.

Discussion. The data of this paper make it clear that pigeons recently exposed to such low temperatures as are normally met with in late autumn usually show abnormal concentrations of reducing substances in the blood, if the concentration found during the summer is taken as the normal. Much the more frequently the blood sugar values are found to be too low after exposure to cold. In most kinds of pigeons the blood sugar apparently returns to the normal after an interval not now definitely known. A notable reduction of sugar values was found 1, 2, 3, 8 and 9 days after a temporary exposure to a temperature of 30°F. and during continued exposure to less severe cold. It is therefore clear that blood sugars obtained from birds recently exposed to cold are not comparable with those not thus exposed; and failure to recognize this factor may introduce serious error into results obtained from pigeons and probably also from many other animals.

Flinn and Scott (7) have studied the immediate effect of higher temperatures during 1 to 6 hours on the blood sugar of dogs. At 20°C. they found during 6 hours a fairly uniform fall in the concentration of the sugar of the blood, but the fall is ascribed to the continued quiet of the dog rather than to the temperature. At 30°, however, this continued decline was scarcely observable beyond the second hour. At 45° and 50°C. during 1 hour, the blood sugar was increased. Hopping (8) found in the alligator a decrease, interpreted as a seasonal variation, in the blood sugar during November–December though the environmental temperature varied but little.

The observed reduction of both blood sugar and ovulation rate as a result of the onset of cold weather supplies an additional instance of this mode of association of the blood sugar and ovulation. It has earlier been shown by Riddle (1) that most ovulations in ring doves are suppressed by the injection twice daily of insulin in doses sufficient to reduce the blood sugar to 62–82 during 1 to 4 hours. We (2) have further shown that close confinement or inactivity during 3 to 5 weeks suppresses ovulation and

markedly lowers the blood sugar. On the other hand, we have made it clear that a temporary increase of the blood sugar occurs in coincidence with the ovulation period in pigeons (3), (4). Parallel with the latter observation Riddle (5), (6) had earlier found a temporary hypertrophy of the suprarenals at the ovulation period. All of these observations suggest that ovulation in birds is normally associated with the capacity of the

TABLE 3

The ovulation rate during the four seasons of the year as found for the 15 ring doves having highest rate of ovulation during the year 1922

NUMBER OF BIRD	NUMBER OF EGGS LAID DURING THE FOUR SEASONS OF THE YEAR									
	January 1 to April 1		April 1 to July 1		July 1 to October 1		October 1 to January 1		January 1 to April 1	
	Total	Singles	Total	Singles	Total	Singles	Total	Singles	Total	Singles
1	22		24		21	1	12		20	
2	19	1	22		21	1	9	1	19	1
3	18		18	2	18		11	1	18	
4	20		22		21	1	5	1	12	4
5	18		18		14		11	1	18	
6	16		18		18		6		14	
7	14		14		22		5	1	14	
8	14		20		18		5	1	14	
9	15	1	19	1	12		10		12	2
10	11	1	19	1	16		7	1	14	
11	10	2	22		15	1	7	1	12	
12	12		18		18		5	1	14	
13	12		18		12		8		12	
14	13	1	16		11	1	4		8	
15	12	2	12		11	1	2		10	2
Totals.....	226	8	280	4	248	6	107	9	211	9
Per cent "singles"...		3.5		2.1		2.4		8.4		4.3

organism to effect a temporary increase of the sugar level beyond the normal, and that conditions which oppose this raising of the sugar level tend to suppress ovulation in pigeons.

The effect of exposure to cold on the blood sugar as here observed by us, and the changes in blood sugar and suprarenal size at ovulation as previously described by us, may next be considered in relation to observations that have been made on the effect of cold on the secretion of adrenin, and to metabolic changes effected by prolonged exposure to cold. Cramer (9) subjected mice to cold and observed that the suprarenals of those mice which best resisted this exposure were well charged with adrenalin, while those that died from such exposure contained little adrenalin. Possibly

the supply of adrenin was here exhausted in the emergency increase of heat production. Very recently Hartman and Hartman (10) have reported that "cooling the skin of an animal (cat) by ice, by immersion in cold water, or by evaporation of water from the skin, causes an increase in output of epinephrin."

In the above observations the time at which the increased adrenin output was found and measured was only a matter of minutes or hours after the application of cold. It seems probable that the increased adrenin output in both of the cases cited above was of too short duration to be really comparable with the periods after cold at which we have found decreased concentrations of blood sugar; that the duration of the hypersecretion continued long beyond the period observed is, however, a possibility. Higher sugar values would ordinarily be expected during the period of an increased adrenin output, but a lower sugar value could result if the rate of sugar combustion were eventually to become disproportionately high. It is further notable that the high blood sugars which we (3), (4) have found associated with ovulation were accompanied by something other than a mere transient secretion—namely, by a hypertrophy lasting several days (5), (6).

Whether the observed reduction of blood sugar and of retarded ovulation rate were accompanied by changes in basal metabolism is, though unanswerable, a question of some importance; for, in one similar and previously described (2) association of suppressed ovulation with reduced blood sugar, there can be little doubt that the metabolism was lowered. In the present case, however, the conditions are those which some investigators consider effective in raising the basal metabolism. On this point it may first be noted that in several communications Boothby and Sandiford (11) have contributed evidence that adrenalin raises the basal metabolism. If this is a fact, and if also the adrenin output under the stimulus of cold is maintained for much longer periods than those already demonstrated by Cramer and the Hartmans and cited above, the presence of an increased metabolism in the birds studied by us is made highly probable. Cannon, McIver and Bliss (12) have very recently suggested that the suprarenals do serve as an important part of the mechanism which prevents the attainment or continuance of too low a percentage of sugar in the blood.

In a report on carefully conducted tests of the effects of out-of-door and indoor conditions upon metabolism Campbell, Hargood-Ash and Hill (13) conclude that "the basal metabolism of the body cells is raised by cool out-of-door conditions, and that shivering is not necessary to raise metabolism. The metabolism is raised by cooling power, not by temperature." Some investigators of the subject, however, do not consider cold, apart from shivering, capable of raising the basal metabolism. One of us has obtained indirect evidence, not easily incorporated here, which is in

best accord with the view that the basal metabolism of pigeons is increased during the approach of cold weather in the autumn. These several considerations suggest—though they do not establish the point—that the suppression of ovulation, along with decreased blood sugar, is sometimes (after close confinement) associated with a lowering of the basal metabolism; and at other times, as in the case of cold weather, with an increased basal metabolism.

SUMMARY

Recent and prolonged exposure to out-of-door air of strong cooling power results in a marked lowering of the concentration of the sugar of the blood of pigeons.

Failure to recognize this effect of cold is or may be a source of error in measurements of the normal sugar level in the blood.

The same temperature conditions which effect a decrease in the blood sugar also diminish the rate of ovulation. At the same time a high percentage of single or "unpaired" ovulations replace normal paired ovulations.

The data supply further evidence that ovulation in birds is normally associated with the capacity of the organism to effect a temporary increase of the blood sugar above its normal concentration, and that conditions which oppose this capacity tend to suppress ovulation.

Some facts suggest that a decrease of both blood sugar and ovulation rate is sometimes associated with a decreased and sometimes with an increased basal metabolism.

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CARDIAC ACCELERATION IN THE ABSENCE OF THE INHIBITORY CENTER

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The question whether cardiac acceleration may occur independently of the inhibitory center is one which has been more or less discussed in the literature for the past twenty-five years or more. However, our views, and especially the views presented in a great many textbooks of physiology, are still somewhat hazy on the subject. In spite of a great many papers in the journals on the subject, it is still an open one, although many experiments have been performed to prove one view or the other. There are two possibilities under which cardiac acceleration may occur in an animal, since there are two mechanisms antagonistic to each other which are at work controlling the heart. While one tends to inhibit the heart and thus keep its rate down, the other tends to accelerate the heart and thus increase its rate. The question then arises which of these two antagonistically different mechanisms causes an increased heart rate? Theoretically, then, we may say that cardiac acceleration may be due to

1. A reflex depression of the inhibitory center or, in other words, inhibition of inhibition.

2. A positive activity of the accelerator center, reflexly or otherwise.

These two views are actually presented to us in the literature and each view has its adherents. Hunt (1) presents the view that cardiac acceleration may occur only through a depression of the vagus action. He claims that reflex cardiac acceleration is caused by inhibition of the cardio-inhibitory center and that he could find no evidence that the accelerator nerves are ever thrown into activity reflexly. He also claims that the most important function of the accelerator nerves seems to be connected with their tonic action; aside from this their function is obscure.

Gasser and Meek (2) claim that the acceleration of the heart at the beginning of voluntary exercise in the normal animal is chiefly due to the decrease in the tone of the cardio-inhibitory center. Their work leads them to believe that the accelerators are a factor of safety and that, in exercise, their action is superimposed on that of the vagi only in time of great need. Aside from this, these authors claim that their function is to maintain the level of the resting pulse.

Roy and Adami (3) and MacWilliam (4) also state that cardiac acceleration may occur only through a depression of the vagus function, and that the accelerators are in no way concerned with cardiac acceleration. A great many more cases might be cited where the conclusion has been reached that cardiac acceleration may occur only through a depression of the vagus function, but the few mentioned above will suffice for the present.

Hunt and other adherents of the first theory presented showed that cardiac acceleration may occur if a sensory nerve is stimulated when the vagi and the accelerators are intact but that no accleration could be shown after the vagi were sectioned and a sensory nerve stimulated. They also showed that, even when the heart was slowed by stimulation of the peripheral end of one of the cut vagi, no cardiac acceleration could be obtained if, at the same time, a sensory nerve was stimulated. Hunt (loc. cit.) also showed that if the accelerators were sectioned with the vagi intact, stimulation of a sensory nerve did give an accelerated heart rate. As a result of all these experiments Hunt and others inclined to the view that cardiac acceleration may occur only through an inhibition of the cardio-inhibitory center.

Opposed to this view is the second supposition that cardiac acceleration may be due to a positive activity of the accelerator nerves reflexly through the accelerator center. This view, too, has many adherents, and only a few of their results will be discussed here.

Bayliss (5) stimulated the depressor nerve in a rabbit after the vagi had been sectioned and obtained a definite increase in the rate of the heart. Since the vagi were no longer present, it would seem that this experimental result was contrary to the view proposed by Hunt and strengthens the view that cardiac acceleration may occur independently of the vagus center.

Hering (6) reaches the conclusion that cardiac acceleration after muscular exercise is dependent on the integrity of the accelerator nerves.

Hooker (7) in a series of experiments on the question of cardiac acceleration reaches the conclusion that there is positive evidence that reflex cardiac acceleration may occur independently of the cardio-inhibitory center. This author also claims that there is a physiological maximum rate of the heart which is usually reached after section of the vagi and beyond which it can only with difficulty be increased. He further states that, if such is the case, the limited amount of evidence in the literature favoring reflex cardiac acceleration through the accelerator center is easy of explanation.

Recently the papers of Wickwire (8), Yates (9), Winkin (10) and Tulgan (11) have brought out some further evidence on the functions of the cardiac nerves, and as a result it seemed advisable to reopen the question of cardiac acceleration.

Experimental procedure. All these experiments were done on cats. The laboratory procedure was ether anesthesia and tracheotomy. Blood pressure and heart rate were recorded by means of a mercury manometer connected to the left carotid artery. Records were made on a Hurthle kymograph. The time was recorded in seconds by a Jacquet chronograph. A signal magnet served as the base line for blood pressure and also indicated by a mark stimulation of a nerve or any other procedure. The heart rate was later computed in number of beats per 10-second intervals. In all cases a control tracing of blood pressure and heart rate was taken before any other procedure was attempted. The depth

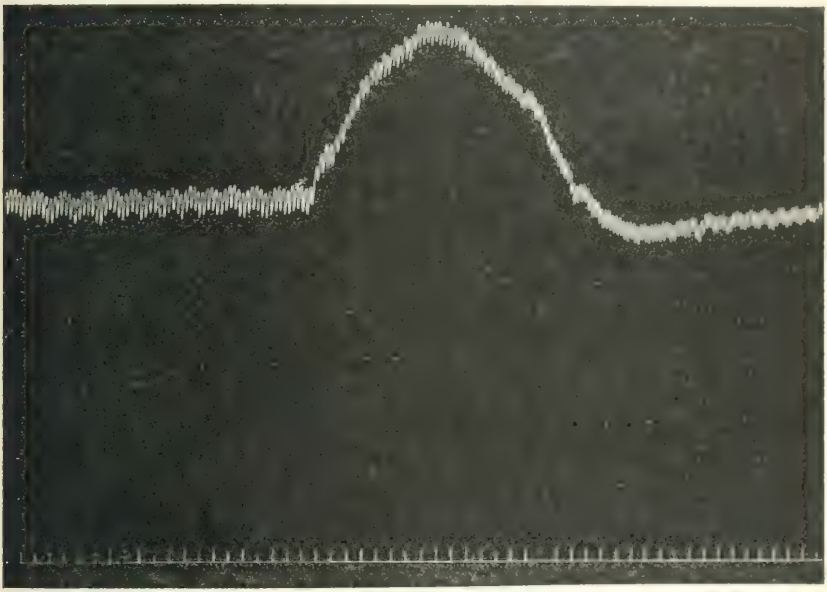

Fig. 1. Stimulation of the central end of the sciatic with the vagi and the accelerators intact.

of anesthesia was uniform throughout the experiment in each case. At all times a sufficient amount of ether was given to abolish all sense of pain in the animals experimented on.

I. Stimulation of the sciatic nerve with the vagi and accelerators intact. After a normal tracing of blood pressure and heart rate was taken, the sciatic, and in some cases the brachial, nerve was divided and the central end stimulated with a medium tetanizing current. A definite rise in blood pressure and an increased heart rate were obtained in every case. Since the vagi and the accelerators were intact, it would be impossible to judge from these results whether cardiac acceleration in this case was due to inhibition of the vagi or to a positive activity of

the accelerators, but when the results obtained in this case are contrasted with the results obtained in the next series of experiments, the value of this experiment will be evident.

	RATE IN 10 SECONDS
Normal	32
Stimulation of the central end of the sciatic, vagi and accelerators intact	40

II. STIMULATION OF THE SCIATIC NERVE WITH THE VAGI INTACT, AND THE STELLATE GANGLIA REMOVED. In this series of experiments the central end of the sciatic nerve was stimulated after the stellate ganglia had been removed. The method used for the dissection of the stellates

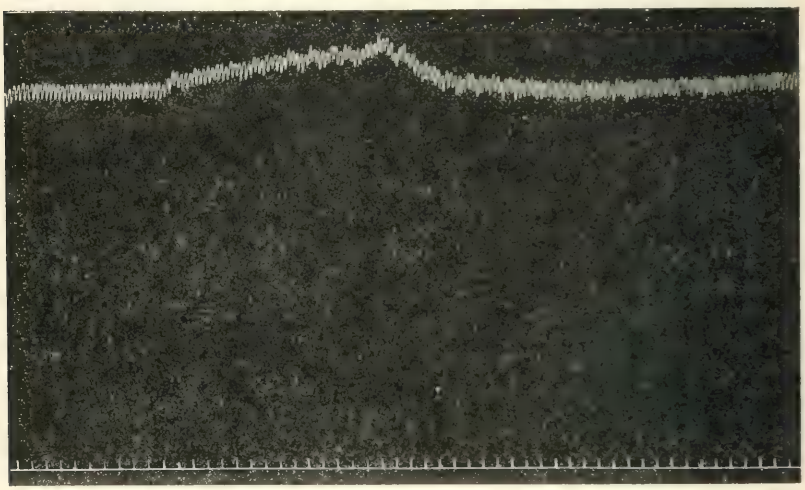

Fig. 2 Stimulation of the central end of the sciatic with the vagi intact and the accelerators cut.

has been described in a previous paper (11) and therefore need not be repeated here. The effect of the stimulation in this case caused a slightly increased blood pressure but there was no evidence of any increase in the heart rate. With the vagi still intact it is difficult to see why an increased heart rate should not be obtained if the theory that cardiac acceleration is due to an inhibition of the inhibitory nerves is correct. Since we do get an increased heart rate with the accelerators and the vagi intact, and do not get any increase in heart rate with the accelerators sectioned, it would seem more likely that an increase in heart rate in this case is due to the accelerators and not to the vagi.

	BEATS IN 10 SECONDS
Normal rate after removal of the stellate ganglia	32
Stimulation of the central end of the sciatic, vagi intact, stellate ganglia removed	32

III. Effect of stimulation of a sensory nerve while at the same time stimulating the peripheral end of one of the cut vagi. If we assume with Hooker that the heart has a physiologically maximum rate which is obtained when the vagi are sectioned, and beyond which rate it is practically impossible to increase it, it is evident that after section of the vagi no increase in rate can be obtained on stimulation of a sensory nerve because the heart is already at its physiological maximum rate. If, however, we can slow the heart up somewhat and then stimulate a sensory nerve we should expect an increase in the heart rate to occur. This was actually done in the following manner. After the vagi had been sectioned, the peripheral end of one of the cut vagi was stimulated and the heart rate thus decreased. At the same time the central end of the cut vagus was also stimulated. The rate immediately increased and stayed higher until the stimulus was removed from the sensory nerve. Immediately the heart rate slowed up again, since the stimulation of the peripheral end of the vagus was continued throughout the period. The same experiment was done, using the central end of the sciatic instead of the central end of the vagus, with the same results.

It would seem from these results that cardiac acceleration may occur through the accelerator center provided the heart is not at its physiologically maximum rate under which circumstances no further acceleration can occur.

Summary. At least 50 experiments were done on cats to determine whether cardiac acceleration is due to a decreased activity of the vagi or to a positive activity on the part of the accelerators. Representative tracings of the three methods of attacking this problem are given. From the results obtained it would seem that cardiac acceleration is due to a positive activity of the accelerator nerves and not wholly to a decreased activity of the vagi.

If a sensory nerve is stimulated, and in these experiments the central end of the vagus, the sciatic and the brachial were used, with the vagi and the accelerators intact, a definite increase in heart rate is observed. This may be due either to a positive activity of the accelerators reflexly through the accelerator center, or to a decreased activity of the vagi reflexly through the inhibitory center. If we assume that the heart rate is at its physiologically maximum rate when the vagi are sectioned, and that we cannot get any further increase on stimulation of a sensory nerve,

then with the vagi intact the heart is not beating at its maximum rate and consequently a definite increase in the rate should be observed on stimulation of a sensory nerve. This increase is actually obtained (fig. 1) and is, as I believe, due to a positive activity of the accelerators, as the next series of experiments shows.

In these experiments a sensory nerve was again stimulated but this time the stellate ganglia were removed previously, and the vagi were left

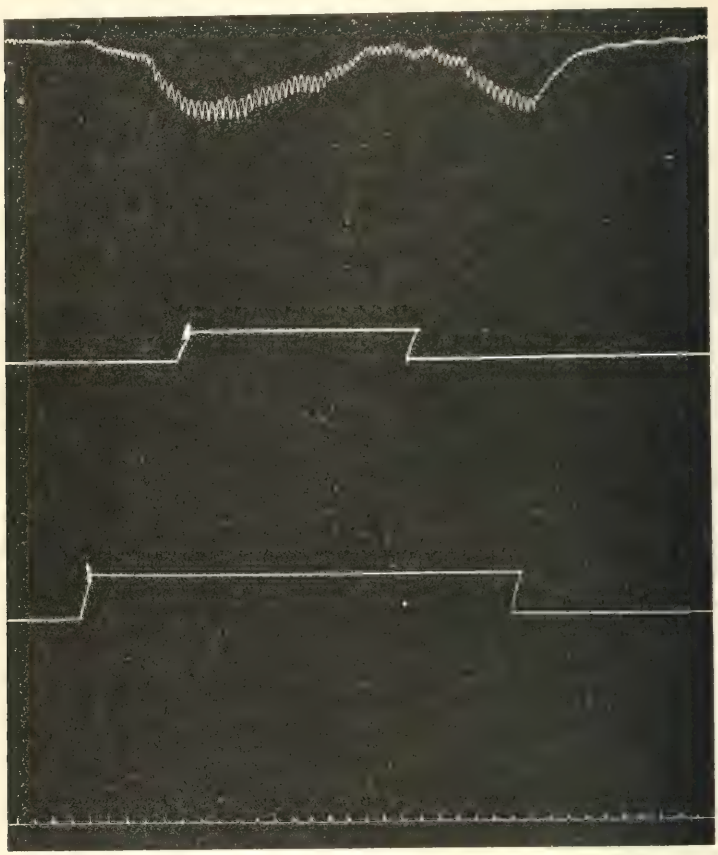

Fig. 3. Stimulation of the central end of the vagus during the period of stimulation of the peripheral end of the vagus.

intact. No increase in heart rate was ever observed. It is difficult to see why cardiac acceleration should occur as a result of inhibition of the vagi with the accelerators intact but why it should not occur with the accelerators removed. This experiment seems to prove that acceleration of the heart with all the cardiac nerves intact is due, not to an inhibition of the vagi, but to a positive activity of the accelerators.

The last series of experiments also proves that if the heart is not beating at its physiological maximum, a definite increase in its rate may be obtained when a sensory nerve is stimulated even when both of the vagi have been sectioned. The heart rate may be decreased by stimulation of the peripheral end of one of the cut vagi. If now the sciatic, the brachial, or the central end of the vagus be stimulated during the time the peripheral end of the vagus is stimulated a definite increase in heart rate is observed. Just as soon as the stimulus is removed from the sensory nerve the heart immediately slows up again. Reference to figure 3 will bring this point out.

One thing[1] seems to have been lost sight of in the discussion so far, or at least to have received insufficient emphasis in the literature. The normal heart rate of an animal rises when the vagi are sectioned, and falls when the accelerators are divided subsequently. The rate falls when the accelerators are divided and rises again when the vagi are sectioned afterward. Theoretically the final rate of the heart should be the same, whatever the order in which the nerves are eliminated. The rate of the heart when all the nerves are cut may be considered as its own autochthonus rate, dependent upon the intrinsic cardiac mechanism. This should be taken as the standard of comparison if we are to get a correct idea of the influences which regulate the heart rate. It should be obvious, also, when the facts are once presented, that inhibition of inhibition cannot, of itself, give rise to a rate any higher than that of the completely denervated heart. The limits of such a change would be that observed when the vagi are divided subsequent to removal of the stellates. It has been shown (8), (11), that the rate of the heart never becomes very high when the vagi are divided following the removal of the accelerators. If the rate of the heart becomes higher than that of the completely denervated heart, some other influence than mere inhibition of inhibition must be operative. The great increase of heart rate after section of the vagi with the accelerators intact can not be considered as due to inhibition of inhibition alone. The increase due to inhibition of inhibition alone would be only that difference between the rate of the heart as observed when all the nerves are intact and the rate which would prevail when all the nerves were cut, and only when the latter is the higher rate of the two.

A concrete example of this may be given. Suppose the observed rate of the heart to be 60. After section of the vagi it rises to 100. After section of the accelerators it falls again to 70.[2] Evidently, only the

[1] This was suggested by Prof. F. H. Pike, to whom I am very much indebted.

[2] Some observations on the rate of the heart of cats after complete elimination of all central nervous influences are given by Stewart, Journal of Experimental Medicine, 1906, viii, 320, and This Journal, 1907, xx, 407.

increase from 60 to 70 can be regarded as due to inhibition of inhibition. The increase from 70 to 100 must be regarded as due to the action of the accelerators. If the heart rate is 60 after section of the accelerators, the vagi being intact, and if it increases to 80 on stimulation of an afferent nerve, the increase from 70 to 80 can not be regarded, without further proof, as due to inhibition of inhibition alone. To regard this increase above 70 as due to inhibition of inhibition is to neglect the accelerator fibers in the vagus (12).

We may sum up the argument by saying that the increase in heart rate above that of the completely denervated heart cannot be regarded as due to inhibition of inhibition, but must be due to some accelerator influence. The clear recognition of these facts should help to clear up much of the confusion in the literature.

CONCLUSIONS

1. An increase in the heart rate may occur independently of the cardio-inhibitory center.

2. Provided the heart is not beating at its physiologically maximum rate, a definite increase in the rate may be obtained by stimulation of an afferent nerve after the vagi have been sectioned.

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ON WEICHARDT'S SUPPOSED "FATIGUE TOXIN"

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Weichardt (1), in 1904, put forth the most remarkable ideas concerning the causation of fatigue that had been suggested since the publication, in 1865, of Ranke's identification of certain "fatigue substances." On the basis of a large amount of experimental work Weichardt claimed that there is formed in fatigue not simply the usually accepted fatigue substances, but a more specific substance, a true "fatigue toxin," analogous to bacterial toxins, and that this is chiefly responsible for fatigue.

A little later than his first experiments he claimed that a substance, or substances, presumably identical with fatigue toxin and possessing similar fatiguing properties, can be obtained from proteins outside the living body, such as egg albumin and the proteins of the press-juice of non-fatigued muscles, by such diverse chemical and physical means as *1*, reduction methods, by sodium sulphite, sodium nitrite and nascent hydrogen, obtained through the agency of colloidal platinum, the negative electrode, and other methods: *2*, oxidation methods, by dilute nitric acid, hydrogen peroxide, the positive electrode, etc.: and *3*, hydrolysis. The product so obtained he named "kenotoxin." He also mentions as sources of kenotoxin urine, the excrement of birds, expired air, plant proteins and bacterial endotoxins.

However obtained, Weichardt claimed that his toxin is capable of forming within the tissues of animals its own antidote, a "fatigue antitoxin," which will prevent the appearance, or, if already present, will eliminate the customary phenomena, of fatigue. Other chemical substances, such as succinimide and certain guanidine derivatives, possess similar antitoxic properties. His belief in the value of his antitoxin, or antikenotoxin, was indicated by the fact that he even went so far as to apply for patents on this product, in Germany in 1903 and in the United States in 1904. The latter was granted two years later, and the official record of the transaction may be found in the report of the U. S. Patent Office (2).

Weichardt has had a few followers, some of whom have presented apparently confirmatory experimental evidence of his conclusions, but some of whose results are fantastic. He has also had certain worthy opponents (3).

In his various publications on fatigue the present senior author's attitude has been one of scepticism of Weichardt's ideas and of a growing belief that fatigue is not a specific phenomenon, caused by a few specific metabolic products, still less by a specific toxin, but that it is a convenient name for the depression of the working power, following any one of many metabolic, or even certain physical, changes in the organism, and that many metabolic substances deserve the name "fatigue substances." We have, therefore, made an experimental test of Weichardt's hypothesis, in so far as it pertains to the production of a fatigue toxin within the animal organism by the performance of muscular work. The reader of Weichardt's successive papers finds occasional contradictions in his statements of experimental results and an ever-increasing complexity of experimental procedure. We have endeavored to simplify the problem and the method and to base our work on the propositions that, if a fatigue toxin exists, it exists, and ought to be capable of demonstration, in the muscles that produce it, and it does not exist in non-fatigued muscles. We published a brief preliminary report of our conclusions in 1917 (4); the presentation of our detailed results has been delayed till the present time. Although our experiments are not very numerous, we believe that the uniformity of the results justifies their publication. Because of our inability to obtain evidence of a natural production of "fatigue toxin" we have not deemed it necessary to proceed to its artificial production or to the production of its antibody.

The method. Our general method has been to fatigue animals, to press out the juice from the skeletal muscles, to filter this juice and inject it into other animals or administer it to excised muscles, and to observe the effects.

As the source of the muscle juice we have used cats chiefly; rabbits were employed in some of the earlier and less satisfactory experiments. The animals were fatigued by being allowed to run in a vertical wheel, 0.914 m. in diameter, constructed of a wooden frame covered by wire netting, the inner circumference forming a running track 2.87 m. long and 20 cm. wide, which was revolved about a horizontal axis by an electric motor. The rate of revolution was adjusted to the disposition of the individual animal to run and varied from 3 times per minute with rabbits to 8 to 15 times with cats. The duration of this fatiguing process varied greatly and its termination was dependent upon a disinclination of the animal to run longer. It was then removed from the wheel and immediately killed by decapitation. By suspension of the body as much blood as possible was made to flow from the severed vessels. In most cases a wire electrode was then placed within the skin around the posterior end of the trunk of the body and another around the ankles, and a tetanic stimulating current was sent through the muscles of the hind legs for

the purpose of causing further fatigue. This was continued intermittently, —usually for from three to ten minutes—until the muscles ceased to contract to the strongest currents that are customarily used for muscular stimulation. The muscles were then removed, cut up by scissors, placed in a mortar, and thoroughly ground with kieselguhr into a pulpy mass. This was folded within several layers of cheese-cloth, placed in a strong steel vise, and subjected to a very heavy pressure. In this manner a quantity of muscle juice was obtained, amounting often to more than 10 cc. This was filtered through filter paper and the juice so obtained was usually employed at once for injection into animals or for administration to muscles as described below. It was always found to be strongly acid in reaction to litmus. The successive processes in obtaining the juice were usually performed aseptically. The time elapsing between death and the completion of the preparation of the juice for injection varied in successive experiments from sixty-three minutes to five hours and eight minutes (in one extreme case), but averaged approximately two and a half hours.

There is no doubt that our animals were thoroughly fatigued in the physiological sense. When taken from the wheel they were breathing very rapidly. Within a short time, often within five to eight minutes, after decapitation the muscles went into pronounced rigor.

The action of muscle juice on animals. We have observed the general action of the juice of fatigued muscles on normal animals. Guinea pigs were usually employed as the test animals, although cats and rabbits have served occasionally. The injections were made sometimes intravenously, sometimes subcutaneously, but in the majority of the experiments intraperitoneally. Usually about 5 cc. of the juice were injected at first, and an additional 5 cc. a few minutes later.

Weichardt observed that when he injected into animals the juice of fatigued muscles, or the artificially prepared kenotoxin, he obtained three distinct effects: stupor, a lowering of the body temperature, and a slowing of the respiration which often ended in death. We have therefore paid especial attention to these phenomena.

In our experiments stupor customarily followed the injection of the juice. The animal became quiet and continued so for hours, often apparently dozing off to sleep.

The most striking effect, however, was a fall of the body temperature. Rectal temperature was observed just before the juice was injected, again usually fifteen minutes after the completion of the injection, and at fifteen-minute intervals thereafter. The fall began promptly after the injection, and the lowest reading of the thermometer was obtained during the first half-hour. Of the ten experiments in which the body temperature was observed, the average maximum fall in the five in which

the observations were most frequent was 1.1° C., the greatest being 1.6°. Immediately after the lowest point was reached the temperature began to rise, and there was a slow gradual return toward the normal. Weichardt reported a continuous fall in temperature and one of several degrees until death usually intervened. In all of our cases there was the fall followed by the rise. Table 1 presents the data from our five most complete experiments. In all of these, cats served as the source of the muscle juice and guinea pigs as the test animals.

Counts were made of the rate of respiration immediately before and at intervals after injection. The results were not constant, there appearing sometimes an increase and sometimes a decrease, and no inference, beyond the fickleness of a rabbit's or a guinea pig's breathing, can be drawn from the figures.

Of nineteen animals injected, five died. Of these three struggled strongly upon the administration of the juice, gave a few gasps and ex-

TABLE 1

Action of juice of fatigued muscles on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS								MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	
	°C.									
15	37.7		0.9		0.7		0.7		0.1	0.9
16	38.6	0.2	0.6	0.5	0.4	0.2	0.1	0	0.6	0.6
17	38.9	1.1	0.8	0.7	0.8	0.4	+0.1	0.1		1.1
18	38.6	1.6	1.4	1.3	1.2	1.1	1.2	1.1	1.1	1.6
22	39.0	0.8	1.2	1.0	1.0	0.8	0.6	0.4	0	1.2

pired at once. In all of these cases the juice was introduced directly into the jugular vein, and the possibility of an embolus was not excluded. In the two other animals, which were injected intraperitoneally, death occurred on the following day, and the autopsy revealed a caked granular mass, apparently the coagulated muscle proteins, surrounding and among the abdominal viscera at the site of the injection.

It is evident that none of the results so far presented are really contradictory of Weichardt's findings or are necessarily opposed to an interpretation of the presence of a fatigue toxin. It should be observed, however, that none of the positive results is specific: Stupor is one of the most common effects of injecting foreign, non-irritating substances into animals; a fall of temperature, especially after the injection of proteins, is well recognized (5); and an occasional death from the introduction of foreign substances into the body through either the intravenous or the intraperitoneal route is to be expected.

The desirability of performing a series of control experiments, in which non-fatigued muscles were used as the source of the juice, seems too obvious

to mention, but a careful search of Weichardt's papers fails to reveal that he performed adequate controls. Rare mention is, however, made of the fact that the juice of fresh muscles proved to be non-toxic or non-fatiguing. We have endeavored to fill this gap, and the significant feature of our work here is that *results similar to those reported above were obtained when the muscle juice from non-fatigued control animals was injected.* This juice was prepared in the same manner as the fatigue juice, except that the animals were not previously fatigued, either by running in the wheel or by the electrical stimulation of their muscles. It was observed that the pulpy mass of muscle, after grinding, was paler and more viscous and yielded juice with greater difficulty than the mass of fatigued muscle. The non-fatigue juice, like that from the fatigued muscles, was acid to litmus. When injected into guinea pigs, there followed stupor, a fall in temperature followed by a rise, and the death of some animals. Table 2 shows the results of the measurements of the

TABLE 2

Action of juice of non-fatigued muscles on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS										MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	
	°C.											
19	38.7	0.8	0.9	0.6	0.6	0.5	0.5	0.6	0.6	0.5	0.4	0.9
20	39.5	2.4	1.9	1.8	1.8	1.3	1.1	0.8	0.4	0.7		2.4
21	39.2	2.2	1.8	1.6	1.2	1.0	0.5	0.6	0.6	0.3		2.2
23	38.3	0.9	1.1	1.4	1.3	1.0	1.1	1.1	1.3	1.1	0.6	1.4
24	37.9	1.5	2.2	1.7	1.2	1.3	0.9	0.8	0.6	0.6	0.3	2.2

temperatures in the five most complete experiments, cats here serving as the source of the muscle juice and guinea pigs as the test animals. It is noticeable here that the maximum fall of temperature is, in general, even greater than with the fatigue juice, the average being 1.8°C. and the maximum 2.4°.

Of the eight animals injected with non-fatigue juice, four died. Of these one, injected by way of the external jugular vein, died immediately afterward, with symptoms of an embolus; two died on the day following the intraperitoneal injection, and one three days afterward. The autopsies in all cases showed conditions not differing from those dead after the intraperitoneal receipt of fatigue juice.

Our observations on the action of muscle juice on animals thus fail to reveal in the juice of fatigued muscles the presence of anything acutely toxic and peculiar only to fatigue. They do justify the assumption, however, that a generally depressing substance is present in both the fatigue and the non-fatigue juice. The continual and fatiguing work of

the animals in the wheel, their excessively rapid respiration and quiet state at the end, and the quick oncoming of rigor after death, are all conceivably explicable on the supposition that in the process of fatigue the muscles received too little oxygen and manufactured lactic acid. The treatment of both the fatigued and the control muscle in the preparation of the juice would only add to the stock of acid in the former and lead to its formation in the latter. It would therefore be expected that both preparations would contain in the end a considerable quantity of acid. Whether the acid alone is responsible for the depression we cannot say, but it is clear that if any single chemical substance is the causative agent it is present in both the fatigue and the non-fatigue juice.

The action of muscle juice on muscles. In an endeavor to demonstrate the action of his supposed "fatigue toxin" on muscles Weichardt performed a number of experiments on mice in which he recorded graphically the contractions of the gastrocnemius muscle in situ. The muscle was stimulated by brief faradic currents every second until exhausted. "Normal curves" were obtained from normal animals; "toxin curves" from other animals which had received a dose of "fatigue toxin" injected into the peritoneal cavity. No mention is made of the way in which such toxin was prepared or of the number of animals of each class used. The published curves show less work performed by the intoxicated individuals, but no computations of the actual amounts of work performed by either class are presented.

We have performed a series of experiments on the action of the muscle juice of both fatigued and non-fatigued animals on muscles. The juice was prepared as already stated, cats being used always as its source. In each test a frog was killed, and its two gastrocnemius muscles were excised, attached to muscle levers, loaded with equal weights, and prepared for simultaneous stimulation by the same single induction shocks and for the recording of the contractions as vertical lines on a slow drum. The skin was left about each muscle and was tied about the tendo Achillis below, thus forming a bag about the muscle, open at the top. The bag surrounding one of the muscles was filled by the muscle juice, which thus continually bathed the muscle tissue. The other muscle received no juice and served for control. The stimulation then proceeded until the two muscles were exhausted. The duration of the work of each muscle was noted, and from the graphic records the total work done by each was computed. The following table 3 shows the effect of the fatigue juice.

It is here seen that with the normal muscle the average duration of the working power was 43.2 minutes and the average amount of work performed 21,039 gram millimeters. For the muscle under the influence of the fatigue juice the average duration of working power was 19.8

minutes, a decrease of 54 per cent, and the average amount of work performed was 10,082 gram millimeters, a decrease of 52 per cent. The depressing action of the fatigue juice is thus very striking.

Before assuming that this result is due to a fatigue substance the above figures should be compared with those of a similar series of experiments shown below, table 4, similarly performed, but in which the action of the juice of non-fatigued muscles was studied.

TABLE 3

Action of juice of fatigued muscles on muscle

NUMBER OF EXPERIMENT	CONTROL GASTROCNEMIUS		GASTROCNEMIUS + FATIGUE JUICE	
	Duration of work in minutes	Work done in gm. mm.	Duration of work in minutes	Work done in gm. mm.
1	90	43,298	33	19,640
2	36	16,560	19	7,695
3	29	12,148	10	3,066
4	23	11,003	12	4,685
5	38	22,189	25	15,327
Average.....	43.2	21,039	19.8	10,082
Percentage.....	100	100	46	48

TABLE 4

Action of juice of non-fatigued muscles on muscle

NUMBER OF EXPERIMENT	CONTROL GASTROCNEMIUS		GASTROCNEMIUS + NON-FATIGUE JUICE	
	Duration of work in minutes	Work done in gm. mm.	Duration of work in minutes	Work done in gm. mm.
6	43	23,030	15	6,271
7	49	22,854	23	13,844
8	46	18,748	20	6,938
9	39	17,023	18	9,227
10	39	20,810	18	11,641
11	33	8,492	17	6,946
Average.....	41.5	18,492	18.5	9,144
Percentage.....	100	100	44	49

Here the surprising fact appears that, while the normal muscle continues to contract approximately as long and to perform approximately as much work as the normal muscle in the previous series—the slight differences being of no significance—the muscle under the influence of the non-fatigue juice loses approximately the same amount as before, namely, 56 per cent in working time and 51 per cent in total amount of work done.

The hypothesis of an acutely toxic substance, present in the fatigue and absent in the non-fatigue juice, is thus no more justified here than in the experiments with the whole animal.

Weichardt's claims may be criticized adversely from several points of view.

In his search for a "fatigue toxin" through the use of animals Weichardt emphasizes the necessity of carrying the fatigue process to an extreme end, complete exhaustion and even death. He thus incurs the criticism of the physiologist, for he demands an artificial condition which is rarely realized in nature—putting an animal to death by overwork is changing a physiological into a pathological phenomenon. But if fatigue be due to the action of a specific "fatigue toxin," then the toxin should be present whenever fatigue symptoms are present, that is, at all times after considerable work, and it ought to be capable of demonstration without the over-fatigue which Weichardt practises. One cannot help suspecting that in his exhausted animals chemical changes have taken place which do not occur in normal fatigue. Moreover, the physiologist may rightly claim that stupor, lowering of the body temperature and slowing of the respiration are hardly adequate criteria of the existence of normal fatigue.

It is only in his earlier work, however, that Weichardt relied upon the fatigue of animals for the production of his supposed toxin. He soon found its chemical preparation more satisfactory. But in reading the accounts of his chemical methods it is difficult to learn exactly what their author was dealing with. It is hardly conceivable that oxidation, reduction and hydrolysis, as employed by him with solutions of protein, would yield chemically identical substances. It is, moreover, not clear whether his products are single chemical substances or mixtures of chemical substances. It is not clear whether he regards all of his so-called "Ermüdungsstoffe," obtained from whatever source, physiological or diversely chemical, as one and the same substance—"toxin" and "toxins" are used indiscriminately in his writings.

Although Weichardt's experiments seem to have been numerous, accounts of them are confined largely to accounts of single selected experiments, and the reader is not offered sufficient information to enable him to form a judgment of the degree of statistical support which his work gives to his conclusions. There is evident, too, a proneness to accept without question favorable evidence, however intrinsically strange. A striking instance of this, which is quoted approvingly by Weichardt, is supplied by Lorentz (6) in his report of the reputed effects of the supposed "fatigue antitoxin" received by inhalation. The rapidity and accuracy of the mental work of certain school pupils were tested by arithmetical problems before and after a five-hour period of instruction. The degree of fatigue resulting was thus measured. After this was ex-

tablished the air of the school room after a morning test was liberally sprayed with a 1 per cent solution of antikenotoxin. The afternoon test then showed that the rapidity of computation was increased by 50 per cent; the number of errors had decreased; and some pupils who were usually sleepy and tired at the end of the period of instruction had a higher grade after than before the five-hours' period! It is not surprising that in an extensive investigation under similar conditions Konrich (7) was wholly unable to confirm these results.

It is conceivable that by Weichardt's extreme treatment of animals and his unique ways of treating proteins outside animal bodies he obtained substances or mixtures of substances that are toxic to the living organism, but he is not justified, we believe, in associating any one of such substances in a specific causal relation with the physical phenomenon of normal fatigue.

SUMMARY

1. When juice is pressed out of the muscles of fatigued animals and injected into other animals there result stupor, no constant effect on respiration, a temporary lowering of the body temperature, and occasionally subsequent death.

2. Such fatigue juice when administered to excised muscles causes a marked decrease in their working power, as shown by a shortening of the working period and a decrease of the total work performed.

3. Juice from the muscles of non-fatigued animals exerts the same action as fatigue juice, both on animals and on excised muscles.

4. The experiments thus fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue.

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ADDITIONAL DATA CONCERNING WEICHARDT'S SUPPOSED "FATIGUE TOXIN"

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Aronovitch and I (1) were unable to confirm Weichardt's claim of the existence of a specific "fatigue toxin." But in our experiments cats, because of their ready fatiguability in our fatigue wheel, were used as the source of the muscle juice, and guinea pigs, because of their docility, were used as the test animals. Thus, by injecting into one species of animal muscle juice obtained from a different species, we incurred the possible criticism that we were merely demonstrating the well-known action of a foreign protein. To obviate this objection, I have performed additional experiments in which guinea pigs were used for both purposes.

The method employed was, in general, similar to that used by Aronovitch and myself, and consisted in thoroughly fatiguing animals, killing them, squeezing the juice from their muscles, and injecting it into fresh animals. For fatiguing the guinea pigs I made use of the treadmill which Hastings (2) had found effective with dogs, and has since been employed by others in this laboratory. It consists of a running-track caged in and propelled by an electric motor at a speed which can be varied according to the running capacity of the individual animal. The guinea pigs, either with or without a few minutes' preliminary training, usually adapted themselves readily to a rate of approximately three-quarters of a mile per hour and were able usually to continue running for more than three hours. In the latter part of the period fatigue became more and more evident, and when the animal ceased to run further, it was removed and immediately killed by decapitation. The blood flowed freely from the vessels and a sample was collected for the determination of its sugar content. The results of such determination will be presented elsewhere. The skeletal muscles were electrically stimulated en masse until they ceased to contract. They were then cut away, freed from connective and fatty tissue, and ground thoroughly in a mortar into a pulp. This was wrapped in cheese-cloth and submitted to very heavy pressure in a strong steel vise. As much juice as possible was thus squeezed out, the amount from a single animal averaging 24 cc. This juice, which was always strongly acid to litmus, was filtered and warmed to body temperature, and a measured quantity of it, usually 10

cc., was injected intraperitoneally into a fresh guinea pig. The effects produced by this injection were then observed. Eight experiments of this kind were performed.

The action of muscle juice on homologous species of animals. Following Weichardt's procedure and as in the experiments with Aronovitch my attention was directed, after injection, to three phenomena: The general behavior of the animal, with especial reference to possible stupor, the rate of respiration, and the body temperature; and these were observed at fifteen-minute intervals during a period of two to six hours. The condition of the animal during several days subsequent to the injection was also studied.

While it can hardly be said that pronounced stupor followed the injection, the animal usually became quiet and mildly soporific, allowing itself to be handled without resistance. In this respect, its behavior was not fundamentally different from that of the animals in the series with Aronovitch, although the soporific effect in the new series was perhaps somewhat less in degree.

As in the series with Aronovitch, the rate of respiration was not affected by the injection in any constant manner. There was much irregularity in the rate, as is not unusual in normal guinea pigs.

The most pronounced and constant effect of the injection was that on the temperature of the body. After the receipt of the muscle juice the temperature, as measured in the rectum, began immediately to fall. The lowest point was reached within a period varying from thirty minutes to two hours, after which the temperature more gradually rose to, or nearly to, the initial point during the period of observation. Of the eight experiments, one, number 66, proved an exception to the rule in showing only minute temperature changes ranging from a preliminary rise of 0.3° to a later fall of 0.2°. Table 1 presents the details of all experiments except number 66, and figure 1 represents the curve of experiment 34, a typical experiment. It may be added incidentally that of the seven animals two died within twenty-four hours and one three days after the injection, while the remaining four continued in good health.

While the effect here is, in general, similar to that in the former series in which non-homologous animals were used, there appear two differences: a much greater average fall of temperature and a slower average recovery. Thus, the possible objection that in the former series the results might have been due to the action of a foreign protein is at once ruled out—whether the muscle juice comes from a different or the same species the sequence of its administration is an immediate fall of body temperature followed by a gradual recovery.

Are we then demonstrating the action of a fatigue toxin? Here again a control series of experiments supplies the answer—a series in which the procedure was in all respects the same as with the fatigue juice save that the

guinea pigs supplying the juice were not fatigued at all. Eight such experiments were performed. There resulted quiet and occasionally real stupor; no constant change in respiration; an immediate fall of body temperature followed by gradual recovery; and the death within twenty-four hours of six of the animals.

The temperature data of seven of these experiments are presented in table 2 and the curve of experiment 53 is plotted as figure 2. Experiment

TABLE 1

Action of juice of fatigued muscles on body temperature of homologous species

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS																				MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	15	30	45	4 hours	15	30	45	5 hours	
	°C.																					
32	37.7	1.1	1.3	1.6	0.9	0.3	0															1.6
33	39.3	2.7	3.3	3.0	2.3	1.9	1.4	1.2	1.0	1.1	0.5	0.5	0.1	0								3.3
34	39.4	1.5	2.2	2.2	2.1	1.9	1.5	1.4	0.8	0.7	0.6	0.3	0.2	0.4	0.4	[illegible]	0.1	0.1				2.2
36	39.4	1.3	1.7	1.6	1.8	1.8	2.1	2.2	2.4	2.2	2.1	2.0	1.8	1.7	1.7	1.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.4
62	39.4	0.2	0.4	+0.1	0.2	0.1	0.2	0.6	0.4	0.5	0.2	0.5	0.6									0.6
64	39.8	0.5	1.2	1.6	2.1	2.2	2.3	2.4	2.6	2.5	2.5	2.3	2.3	2.1	2.0	2.0	2.1	2.0	2.0	1.8	2.0	[illegible]
67	38.9	1.4	2.3	2.5	2.7	3.0	3.3	3.3	3.3	3.0	2.8	2.6	2.4	2.2	1.9	1.5	1.1	1.1	0.6	0.3	+0.3	3.3

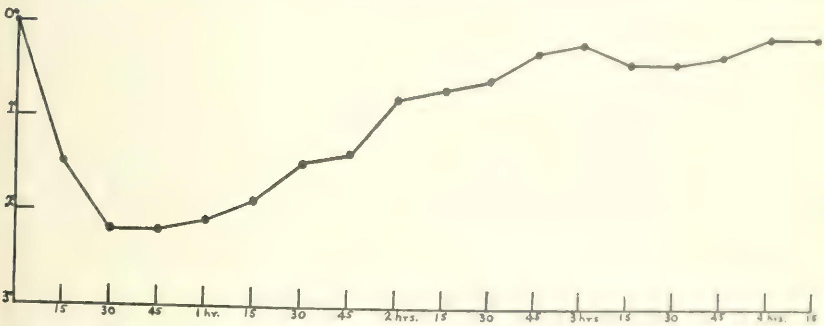

Fig. 1. Action of juice of fatigued muscles on body temperature. Experiment 34, table 1.

38 showed a fall of temperature so far in excess of all the others—more than five degrees and so far beyond the minimum limit of the clinical thermometer as to be incapable of measurement—that it has been omitted from the published records.

Here again the tale is repeated: as in the experiments with Aronovitch non-fatigue juice produces the same effect as fatigue juice. This is true whether the recipient of the juice is of the same species of animal as the donor, or of a different species.

Enough evidence has now been presented, I believe, to make it clear that Weichardt's assumption of the existence of a specific toxin of fatigue quite lacks a reliable experimental basis and is quite needless to account for the well-known facts of fatigue.

The action of lactic acid on animals. Since the muscle juice in both the above series was always strongly acid to litmus, I was curious to know whether its physiological action might be due to its acid content. This question had been raised in the paper by Aronovitch and myself, but we did

TABLE 2

Action of juice of non-fatigued muscles on body temperature of homologous species

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS																						MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	15	30	45	4 hours	15	30	45	5 hours			
	°C.																							
35	38.8	1.1	1.2	1.4	1.3	1.0	0.8	0.9	1.0	1.0	0.9	0.9	0.5	0.6	0.7	0.4	0.4	0.2	0.3	0.2	0.2			1.4
37	39.3	0.8	0.9	0.8	1.0	1.1	1.3	1.2	1.4	1.2	1.0	0.8	0.7	0.6	0.5	0.6	0.5	0.5						1.4
39	39.8	1.1	1.4	1.4	1.0	1.0	0.8	0.5	0.6	0.9	1.1	1.2	1.2	0.8	0.8	0.4	0.4	0.2	0.2	0.2	0.5			1.4
51	38.9	0.3	1.4	1.5	1.7	1.5	1.5	1.3	1.3	1.3	1.5	1.2	0.9	1.1	0.7	0.7	0.9	0.4	0.3	0.3	0.3			1.7
53	38.0	1.2	1.6	2.2	2.2	1.8	1.0	0.6	0.3	0.1	0													2.2
59	39.6	1.1	1.8	2.3	2.8	3.2	3.3	3.1	3.1	3.0	2.8	2.3	2.1	1.8	1.5	1.3	1.1	1.0	0.9	0.8	0.8			3.3
60	39.1	0.9	1.2	1.8	2.0	1.9	2.1	2.2	2.4	2.4	2.4	2.3	2.2	2.4	2.3	2.1	2.0	1.9	2.0	1.9	1.7			2.4

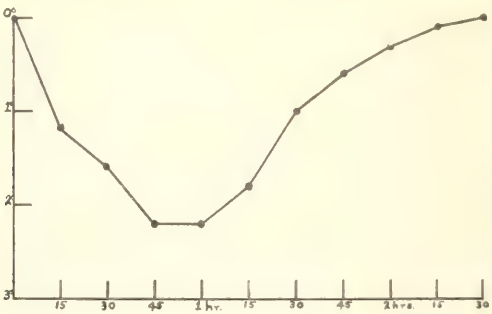

Fig. 2. Action of juice of non-fatigued muscles on body temperature. Experiment 53, table 2.

not attempt to answer it experimentally. I have therefore made a few experiments bearing upon it.

Dr. C. D. Murray courteously determined the pH in two specimens of juice, one from non-fatigued and one from fatigued guinea pigs, and found that of the former to be 5.6 and that of the latter 5.8. Through the courtesy of Dr. F. B. Flinn, determinations of lactic acid were made in four specimens each of non-fatigue and fatigue juice. The figures, expressed in milligrams of lactic acid in 100 cc. of juice, were: for the non-

fatigued animals, 222, 198, 212, 210, the average being 210; and for the fatigued animals, 223, 207, 197, 247, the average being 218.

Seven healthy, non-fatigued guinea pigs were injected intraperitoneally, each with 10 cc. of a solution of lactic acid consisting of 220 mgm. in 100 cc. of an 0.9 per cent solution of sodium chloride and warmed to body temperature. Following the injection the animals became very quiet, sometimes relaxed muscularly, sometimes trembling as if feeling a chill; there was no observable effect on respiration; there were no deaths. But there was an

TABLE 3

Action of lactic acid on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS												MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	
	°C.													
41	39	0.7	0.8	0.8	0.5	0								0.8
42	38.7	1.1	1.7	1.5	1.2	1.1	0.7	0.2	0					1.7
49	39.2	1.0	1.1	1.2	1.2	1.3	0.9	0.7	0.5	0.5	0.4	0.2	0	1.3
52	38.3	0.1	0.5	0.6	0.4	0.4	0.3	0.1	0					0.6
56	39.2	0.1	0.4	0.2	0.2	0.1	0							0.4
57	39.5	0.6	0.5	0.7	0.7	0.5	0.3	0.4	0.3	0.3	0.5	0.2	0.4	0.7
58	39.7	0.8	0.9	1.6	1.4	1.2	1.0	0.7	0.6	0.6	0.1	0		1.6

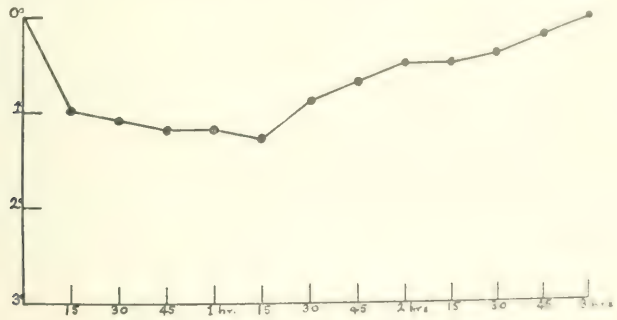

Fig. 3. Action of lactic acid on body temperature. Experiment 49, table 3.

immediate fall in body temperature, followed by gradual recovery. The data on body temperature are presented in table 3 and the curve of the typical experiment 49 is plotted in figure 3.

Still again we see presented the familiar picture. The effect is not so profound as with the muscle juice, but it is of the same sort. It may therefore reasonably be inferred that the temperature-depressing action of muscle juice, whether from fatigued or non-fatigued animals, is due in part at least to their content of lactic acid.

SUMMARY

1. When juice is pressed out of the muscles of fatigued guinea pigs and injected into fresh guinea pigs there result quiet, no constant effect on respiration, a temporary lowering of the body temperature and occasionally subsequent death.

2. Juice from the muscles of non-fatigued guinea pigs exerts the same action on fresh guinea pigs as fatigue juice.

3. Lactic acid, when injected into guinea pigs, is followed by quiet and a temporary fall of body temperature, which is less in extent than that following the injection of muscle juice.

4. The experiments fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue.

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APPLICATION OF THE DIFFUSION HYPOTHESIS TO MEMBRANE POTENTIALS.

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In a paper on the acid-base equilibrium in two phase systems[1] the following case was suggested as an interesting one from the point of view of the Donnan equilibrium.

Two aqueous solutions, A and B, contain equal concentrations of undissociated lactic acid (HL), but different concentrations of Na-lactate (NaL). The solutions are separated by a layer of amyl alcohol in which there is a sufficient concentration of HL to be in equilibrium with both aqueous solutions, but in which the NaL is practically insoluble. The condition is approximately the one which would be obtained if amyl alcohol containing HL, in excess of the total base present, were shaken up several times alternately with two aqueous solutions containing originally two different concentrations of NaOH. The conditions of this equilibrium and the limitations of the mathematical treatment are fully described in the paper above.

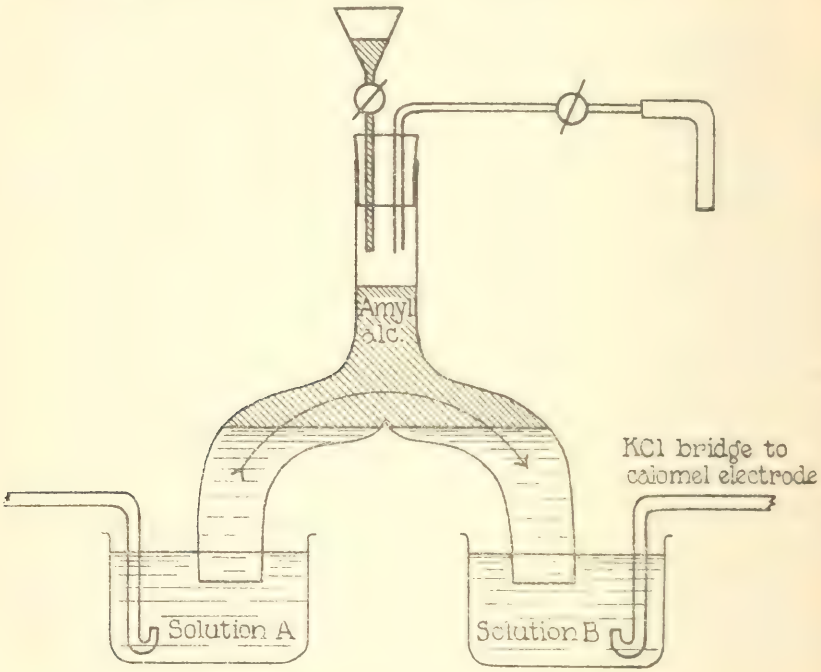
In either solution (A or B) the greater part of the lactate ions (L) are derived from the Na lactate, and the hydrogen ion concentration (except in the very acid solutions) can be calculated from the equation

$$H = \frac{HL}{NaL} \times \frac{K}{\gamma}; \text{ or } pH = \log \frac{NaL}{HL} + pK' \qquad (pK' = 3.85)$$

At equilibrium, $HL_A = HL_B$, therefore $H_A \times L_A = H_B \times L_B$. Hence the solution with the lower concentration of NaL will have a lower concentration of L and a higher concentration of H. The conditions of the Donnan equilibrium in this respect are obviously fulfilled. The amyl alcohol is as effective a semipermeable membrane as can or

[1] Murray, C. D., *J. Biol. Chem.*, 1923, lvi, 569.

dinarily be used in an actual experiment. In this case it is relatively impermeable to Na ions, which here play the rôle of the protein ions often used in similar studies with collodion membranes. It will be noted that in this case the relative indiffusibility of the Na ions is a condition imposed by the membrane, whereas in experiments with protein ions these ions are inherently almost non-diffusing, and therefore in many cases the membrane hardly alters the nature of the system.



Arrow shows direction of diffusion current when sign of P.D. is positive.

FIG. 1.

EXPERIMENTAL.

With the system described above several series of experiments were performed to determine the potential differences between various pairs of solutions, made up so that the condition of equilibrium de-

scribed would be satisfied. The P.D. was measured by a potentiometer in the usual manner with the use of two saturated calomel electrodes.

The *sign* is taken as positive if the direction of the positive current is from left to right through the *solutions* (as drawn or described).

All the aqueous solutions had a concentration of 0.05 N HL, with varying amounts of NaL in addition. The pH of each solution was measured electrometrically.

Solution A, the most acid of the series and containing the least amount of NaL, was placed in the beaker on the left, and Solution B on the right, and the tips of the calomel electrodes were inserted. Through the top of the A-tube, which is shown in the drawing, amyl alcohol containing 0.025 N HL (equilibrium concentration previously determined) was introduced. Then at the top of the A-tube suction was applied until the levels of the two aqueous solutions nearly reached the ridge, leaving, therefore, a small portion of amyl alcohol as a junction or membrane.

At this point the P.D. was measured. This will be designated the *alcohol junction* P.D. Finally further suction was applied to allow the two aqueous solutions to come in direct contact by just surmounting the ridge and regulating the levels so that the less dense solution surmounted the ridge first. The *aqueous junction* P.D. was then quickly measured. By lowering and raising the levels again the measurements could be repeated several times with variations within 3 mv.

Introducing fresh portions of Solution A, fresh alcohol, and substituting successively Solutions C to I in place of B, a series of P.D. was obtained for the alcohol and aqueous junctions (Series 1). Solution C was then substituted for Solution A and measured against Solutions D to I (Series 2). Finally, Solution D was measured against Solutions E to I (Series 3).

The results are given in the table. Under Series 2 there are two columns, the first gives the observed P.D. figures in millivolts as measured, and the second gives the sum of the P.D. referred to Solution A. (Thus, A *vs.* C + C *vs.* I = 63.3). Series 3 is similarly treated. It will be seen that the P.D. is nearly additive.

In addition to the series tabulated, 0.01 N NaL was measured against 0.1 N NaL, thus eliminating the HL.

0.01 NaL *vs.* 0.1 NaL (alcohol junction) = 12 mv.
0.01 " " 0.1 " (aqueous ") = −5 mv.

The results of the last mentioned experiment are indicated in Fig. 2 by the dotted slopes. It will be evident that a difference in pH of 1 in Series 1 is very nearly equivalent to a difference in pL of 1 in this last experiment. The other curves in the figure represent the P.D. obtained in Series 1, *i.e.* between Solution A of pH 2.50 and the solutions having the pH indicated by the abscissæ. The steep slope indicates the theoretical P.D. if the system followed the Donnan conditions exactly.

TABLE I.

	Solution.	pH	Series 1.	Series 2.		Series 3.	
Alcohol junction.	A	2.50	0.0				
	B	2.68	10.2				
	C	2.94	19.3	0.0	(19.3)		
	D	3.65	44.5	24.5	43.8	0.0	(44.5)
	E	3.97	47.7	31.5	50.8	3.6	48.1
	F	4.30	—	39.0	58.3	—	—
	G	4.66	54.0	44.0	63.3	13.0	57.5
	H	5.08	59.0	43.0	62.3	14.2	58.7
	I	5.50	61.0	44.0	63.3	20.0	64.5
Aqueous junction.	A	2.50	0.0				
	B	2.68	1.0				
	C	2.94	6.0	0.0	(6.0)		
	D	3.65	3.0	—	—	0.0	(3.0)
	E	3.97	0.2	—	—	−2.2	0.8
	F	4.30	—	—	—	—	—
	G	4.66	−5.2	—	—	−4.5	−1.5
	H	5.08	−7.5	—	—	−10.0	−7.0
	I	5.50	−13.0	−17.0	−11.0	−14.0	−11.0

THEORETICAL DISCUSSION.

Let us first summarize the specific properties of the system with which we are dealing. Let x and y be any two solutions such as were described in the beginning. Then, within the limits set forth in the previous article

$$HL_x = HL_y$$
$$H_x \times L_x = H_y \times L_y$$
$$pH_y - pH_x = pL_x - pL_y$$

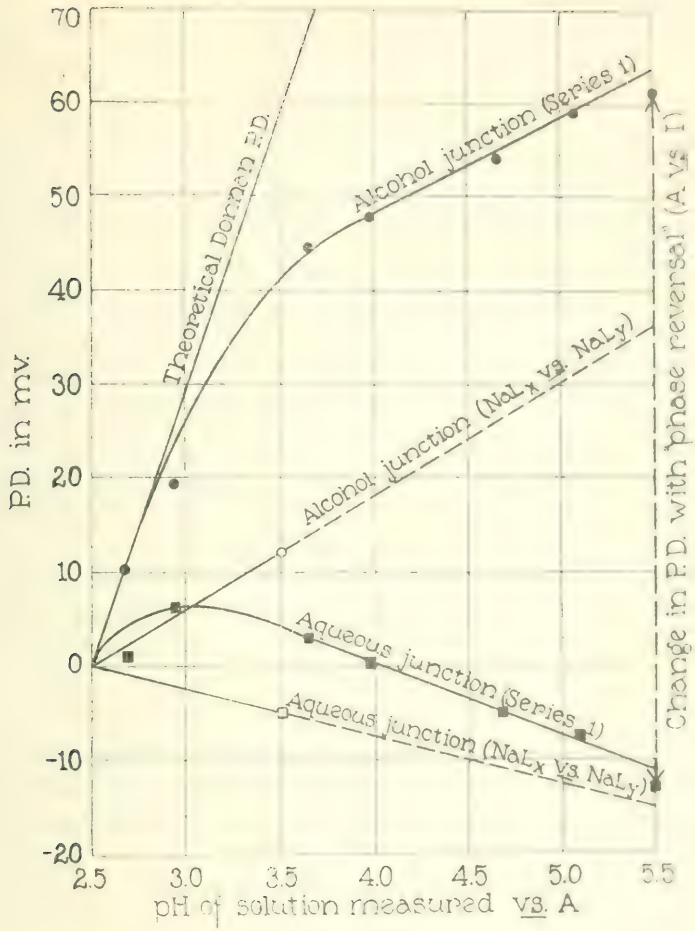


FIG. 2.

and in all but the most acid solutions, since

$$Na_x = L_x \text{ and } Na_y = L_y \text{ (very nearly)}$$

then

$$pH_y - pH_x = pNa_x - pNa_y$$

$$\frac{H_x}{H_y} = \frac{L_y}{L_x} = \frac{Na_y}{Na_x}$$

or

$$-\Delta pH_{(x-y)} = \Delta pL_{(x-y)} = \Delta pNa_{(x-y)}$$

In the experiment with the two concentrations of NaL, we have merely

$$\Delta pNa = \Delta pL$$

From these data we can write the classical approximate equations for two simple cases of diffusion potentials.[2]

A. When the concentration of H is negligible compared to the other ions, we have

$$E_{(x-y)} = \frac{M_L\,(L) - M_{Na}\,(Na)}{M_L\,(L) + M_{Na}\,(Na)} \times 59 \log \frac{Na_y}{Na_x} \qquad (1)$$

in millivolts, where M_{Na} and M_L represent the ionic mobilities of the respective ions, and (L) and (Na) represent the mean concentrations of these ions in the region of the diffusion boundary. The term M_L (L) evidently represents the mean specific conductivity of the lactate ions, etc. This is the familiar equation for the diffusion potential between two concentrations of the same salt.

B. When the concentration or the mobility of Na is reduced to zero at the boundary due to an impermeable membrane, we have

$$E_{(x-y)} = \frac{M_H\,(H) + M_L\,(L)}{M_H\,(H) + M_L\,(L)} \times 59 \log \frac{H_x}{H_y} \qquad (2)$$

which is the same as the familiar equation for the diffusion potential which can be obtained between two solutions satisfying the Donnan equilibrium. Unfortunately, this equation can be simplified to

$$E_{(x-y)} = 59 \log \frac{H_x}{H_y} \qquad (3)$$

I say "unfortunately," because many who have used equation (3) have not emphasized the derivation which Donnan used. The distinction between the conditions of equation (1) and equation (2) is that:

In the first case, the positive and negative ions tend to diffuse in the *same* direction and the net E.M.F. depends on the *difference* in their transport numbers.

[2] The derivation of the Donnan P.D. is given in Lewis, W. C. McC., A system of physical chemistry, London, 1922, ii, 320. Simple diffusion potentials are treated on p. 151.

In the second case, the positive and negative ions tend to diffuse in opposite directions and the resulting E.M.F. depends on the *sum* of their transport numbers (the sum, of course, equals 1).

Interpretation of Experimental Results.

Now in Series 1, with the alcohol junction, between the first three or four solutions equation (2) holds as the limiting condition—we have a distinct Donnan P.D.

In the experiments with two concentrations of NaL (no HL), we have a case of a simple diffusion potential and applying equation (1) it will be noted from the sign that the term $M_{Na}(Na)$ is greater than $M_L(L)$ in the *aqueous* junction, whereas in the *alcohol* junction the term $M_L(L)$ is the greater. Roughly, $\frac{M_{Na}(Na)}{M_L(L)} = 1.2$ in H_2O, $=0.66$ in amyl alcohol, when $Na_x = L_x$, and $Na_y = L_y$. The results obtained between the last few solutions of Series 1, *i.e.* Series 3, follow the slopes indicated by the experiments with NaL solutions with the corresponding junctions and can evidently be interpreted along the lines suggested by equation (1).

But taking the alcohol junction P.D. of Series 1 over the whole range, as we measure Solution A against solutions of increasing NaL concentrations we are involved in a transition from the conditions of equation (2) (the Donnan P.D.) to those of equation (1) (the simple diffusion P.D.). Though the solubility of NaL in amyl alcohol is small, as the concentration of NaL in the aqueous solution increases, the specific conductivity due to NaL in the alcohol tends to outweigh the conductivity of the dissociated HL. As the conductivity of H ions in the boundary becomes relatively negligible we approach the conditions of equation (1).

In the case of the aqueous junctions there is at first in Series 1 a slight Donnan effect, (because the solutions are originally made up to satisfy a Donnan equilibrium as if they had been separated by an amyl alcohol membrane impermeable to Na) but obviously the increasing NaL concentrations outweigh the dissociated HL much sooner in these cases than in the cases with the alcohol junctions.

In themselves these experiments support the view advocated by Cremer[3] and more recently by Prideaux and Crooks.[4] They have convinced me that it would be more profitable to recognize the potentials occurring in such systems to be indications of a definite tendency of ions to *diffuse* from a region of high activity to one of lower activity. In all such cases, whether we have gelatin chloride in a collodion bag or NaL solution in contact with alcohol, the supposition is that (at equilibrium) the activities of the "undissociated" molecules

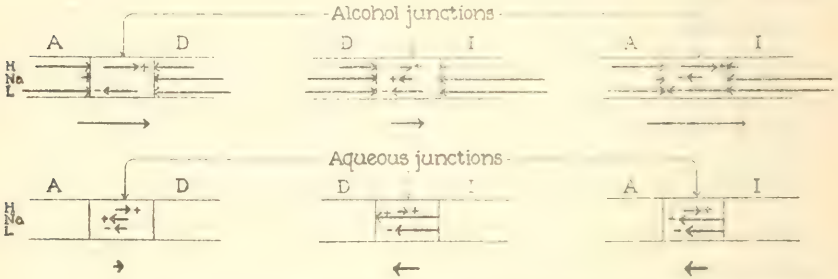


FIG. 3. These diagrams picture the diffusion process occurring in the various boundaries. The arrows in the end sections of each diagram represent roughly the concentrations in logarithmic units of H, Na, and L ions in the solutions corresponding to the letters. The bottom row, in this respect, is the same as the top row. The arrows in the central sections represent the relative tendencies to diffuse of the H, Na, and L ions. The arrow under each diagram represents the net P.D. and the direction in which the positive current tends to flow. The first diagram in the top row represents the conditions resulting from a previously established Donnan equilibrium. The central diagrams of both rows, if the H ion is neglected, represent simple diffusion conditions such as exist between two concentrations of the same salt. The other diagrams represent combinations of the two simple cases.

which can exist in both phases and migrate from one to the other must be equal in both phases so long as there is not a complete electrical circuit or a dissymmetry of membranes. This results, frequently, in the condition in which the activities of an individual ion differ in the two phases, although the activity product of the two ions which form the undissociated substance still remains equal in both phases.

[3] Cremer, M., *Z. Biol.*, 1906, xlvii, 562.

[4] Prideaux, E. B. R., and Crooks, W. E., *Chem. and Ind.*, 1923, xlii, 955.

Then, when the electrical circuit is completed, ions can move independently and the tendency is for the activities of individual ions to become equalized if the electrode P.D. is the same in the two phases (supposedly zero for saturated calomel electrodes). It is understood that, at equilibrium, what we have measured and called a diffusion potential is, under conditions where electrodes are not present, balanced by the electrostatic forces which hold oppositely charged ions together. These P.D. have usually called attention to the electrostatic condition, and the fact has been lost sight of that the source of the P.D., the source of the current (which we oppose with a battery or a charged electrometer) is due to the diffusing tendency of the ions, although this fact was well brought out by Donnan. Much has been written about "electrical double layers" and "charges" have been referred to as if they existed in aqueous solutions independently of ions. Some, too, have spoken of glass bulbs and collodion membranes acting "as hydrogen electrodes" merely because observed P.D. are proportional to the differences in pH on the two sides. This relation holds as long as the conditions of a true Donnan equilibrium are satisfied, but breaks down experimentally if the membrane becomes permeable to the ion which had been considered indiffusible, which happens usually when the concentration of this ion is large. Obviously everyone is referring to the same phenomenon, but I believe that the "kinetic" interpretation describes the processes in simpler terms and leads us more directly to an understanding of the discrepancies and anomalies in experimental results. The problem resolves itself to this general question:

A membrane, or a metal electrode, or an amalgam, or a piece of glass, all involve a second phase when in contact with a solution; what then are the thermodynamic properties of this second phase? At equilibrium what are the activities, the distribution coefficients, and the mobilities of the various components of the system in this second phase?

Incidentally, it might be mentioned that the experiments, as carried out, form an interesting example of "phase reversal." Changing from the alcohol to the aqueous junction in the last experiment listed (A *vs.* I) caused a change in P.D. from +61 mv. to −13 mv. It will be noted that in experiments with gelatin solutions removal of the collodion membrane causes no essential change in P.D. (in many cases

because the gelatin ions are inherently non-diffusing. Many combinations of these experiments can be devised and membranes operated to simulate action currents and other bioelectric phenomena.

SUMMARY.

A system consisting of two aqueous solutions, containing equal concentrations of lactic acid, but different concentrations of Na lactate, separated by a layer of amyl alcohol has been described. This system exhibits electrical properties ranging (as the concentration of NaL is increased) from those characteristic of a simple Donnan equilibrium to those characteristic of simple diffusion.

The fact that the Donnan P.D. can be treated as a special case of a diffusion potential has been emphasized.

The experiments call attention to the effect of the thermodynamic properties of the membrane, and it is suggested that such properties as conductivities, ionic mobilities, and distribution coefficients in membranes of any sort should be investigated.

The experiments afford an interesting example of "phase reversal."

PRIMARY SARCOMA OF THE STOMACH

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PRIMARY SARCOMA OF THE STOMACH*

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That primary sarcoma of the stomach is rare has been recognized by our pathologists. Several writers have collected reports of these tumors. In 1900, Fenwick [1] collected sixty cases in the literature and concluded that fifty-three were authentic. Flebbe,[2] in 1914, collected 157 cases. Haggard [3] collected 244 cases in 1920.

The rarity of these tumors is readily seen from this list: One sarcoma of stomach in 840 specimens (Berlin Pathological Institute), six in 13,387 necropsies (Hirsch), and eight in 2,069 malignancies of stomach (Mayo clinic during five years).

Ewing [4] has stated that sarcoma of the stomach constitutes 1 per cent. of gastric tumors.

The following case came under my observation while in charge of the first medical division pathological department of Columbia University at Bellevue Hospital.

REPORT OF CASE

G. D., a man, aged 77, Roumanian, single, was admitted to hospital Jan. 12, 1923, with a history of weakness for the last three months. He had had gonorrhea and syphilis (fifty years ago), pneumonia and furunculosis (two years ago). There was no history of any injury. His father and mother had died of "old age." One brother was living and well and another had died of heart trouble. There was no history of any chronic disease in the family, or tobacco or alcohol.

Three months previous to admittance the patient became unconscious in the street. The onset was sudden, with weakness. There was no paralysis or injury. He was taken home and became conscious, with the intense weakness continuing. His appetite had been poor for three months, although there was no distaste for food. There was no vomiting, headaches or abdominal pain. There was burning in the epigastrium of four months' duration, with difficulty in swallowing. The patient had been losing considerable weight for several years. He occasionally had a productive cough. The bowels were constipated. There was no pain. He rose once during the night to urinate, otherwise his sleep was fair. His vision was poor and the left eye was blind. He had weighed 220 pounds (99 kg.) and now weighed 175 pounds (74.9 kg.). The loss had been rapid.

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1. Fenwick: Primary Sarcoma of the Stomach, Lancet **1**:463 (Jan.) 1901.

2. Flebbe: Ueber das Magensarkom, Zeitschr. f. exper. Path. u. Therap. **12**:311-336, 1913.

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At the time of examination the patient was lying quietly in bed and looked chronically ill. The conjunctivae were slightly injected, and there was a cataract of the left eye and arcus senilis. Several teeth were missing, several had been filled and the remainder were carious with slight pyorrhea beginning. The tongue was dry and coated, protruding in the midline. There were no abnormal masses or pulsations in the neck. The chest was symmetrical but hyperresonant throughout. The pulse was small and regular with sclerotic vessels. The heart was enlarged to the left and the sounds were poor in quality. The abdomen was slightly retracted and a mass was felt in the epigastrium. The liver was palpable, three finger breadths below the costal margin. The skin was dry and atrophic and there was moderate adenopathy. There were slight petechial edema of both legs. The blood pressure, taken on the right arm, was: systolic 105, diastolic 65. The urine was acid with no albumin and a specific gravity of -,020. The stool was black and gave a positive guaiac reaction. The red cell count was 2,800,000, the white cell count was 16,000 and the hemoglobin was 45 per cent. The nonprotein nitrogen in the blood was 48 mg., the creatinin

Fig. 1.—The stomach, showing the sarcoma in the cardiac region.

1.8 mg., and the uric acid 2.26 mg. The patient died Jan. 16, 1923. The diagnosis was as follows: neoplasm of the stomach; arteriosclerosis (general); chronic myocarditis; senility.

Necropsy.—The body was that of a well developed white man, about 78 years of age, 5 feet 9 inches (164 cm.) in height. The skin was much wrinkled. The hair distribution was normal. There were no noticeable changes in the external nares, mouth, ears or eyes. The external genitalia were normal.

Abdomen.—A midline incision was made in the trunk in front. The peritoneum was smooth and glistening. The diaphragm extended upward to the fifth interspace on the right, and to the upper margin of the sixth rib on the left.

Chest.—There was considerable yellow fat over the pericardium. The pleural cavities were dry. The lungs were normally free.

Heart.—The visceral and parietal layers of the pericardium were smooth and glistening, and contained the normal amount of straw colored fluid. The heart was normal in size. In the right side of the heart there was a small quantity of deep red, partially clotted and fluid blood. The auriculoventricular junction admitted the entrance of two fingers. The endocardium of the right side of the heart was well preserved and the pulmonary valves and pulmonary artery were normal. The endocardium of the left side of the heart was normal. The mitral

valve was unchanged. The aortic valves were slightly thickened. The surfaces made by sectioning the heart muscle were dark red.

Lungs.—These were easily removed. They were large, of normal weight and they crepitated throughout. On the surface of both lungs there were mottled white and black regions. These black regions were more marked at the apices. The surfaces made by sectioning had a few white nodules, the size of a pinhead, firm, and extended slightly over these surfaces. The mucosa of the large bronchi was normal. The peribronchial lymph glands were not enlarged.

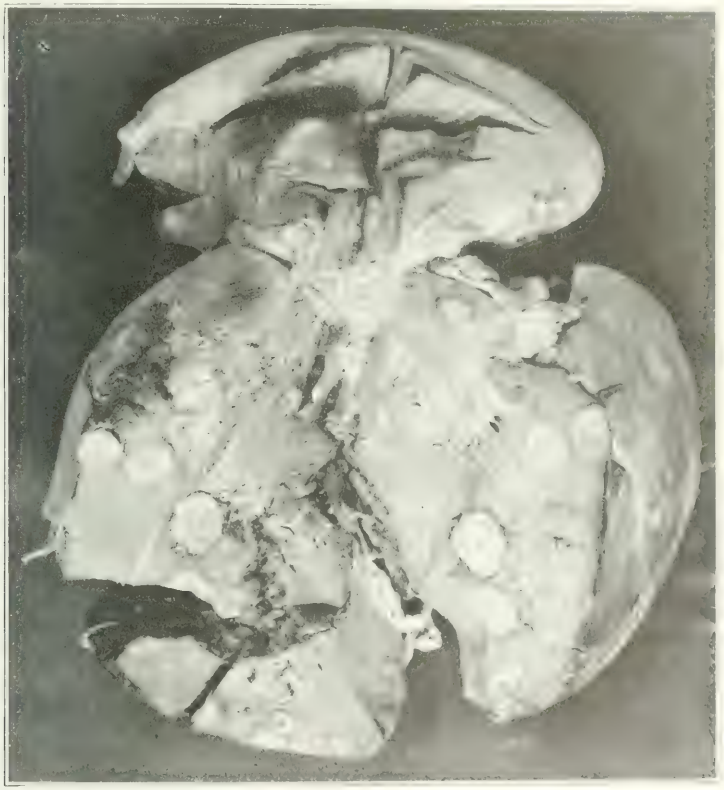


Fig. 2.—The liver with the metastatic nodules of the sarcoma.

Spleen.—There was no noteworthy gross pathology shown.

Pancreas.—It was small and pink. The surfaces made by sectioning were normal.

Suprarenals.—They were small. The cortices were narrow and yellow. The medullae were smooth and gray.

Kidneys.—They were small and embedded in fat. Their capsules were thickened, and stripped with difficulty, leaving a granular surface. They did not cut easily. The cortices and medullae were not well differentiated and the cortices were contracted. The pelves contained a small amount of fat.

Urinary Bladder.—It was slightly distended with amber colored urine. The mucosa was pale. The openings of the ureters were patent.

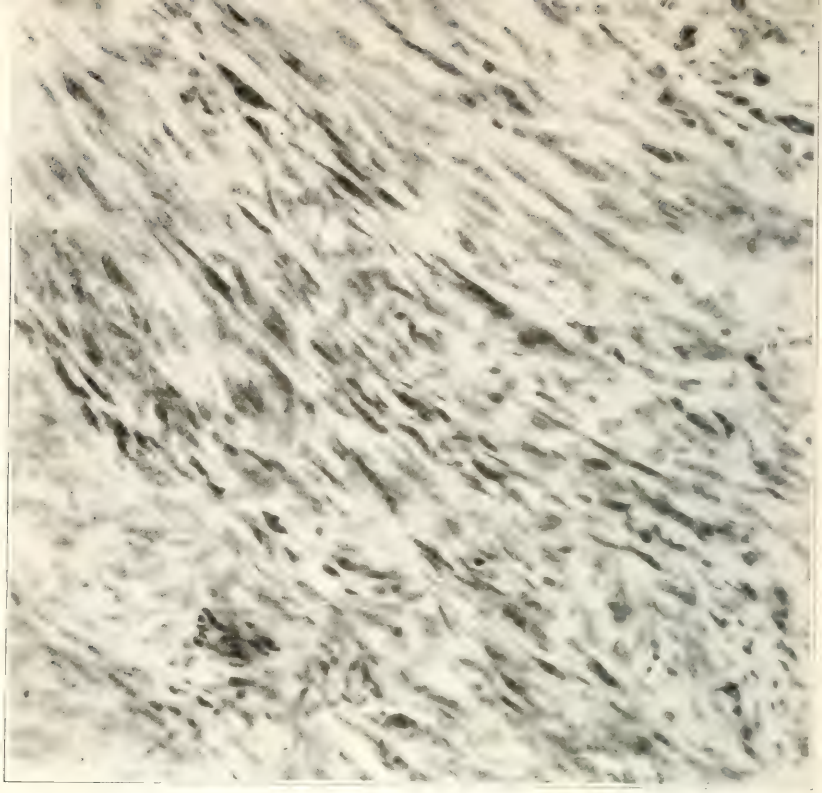
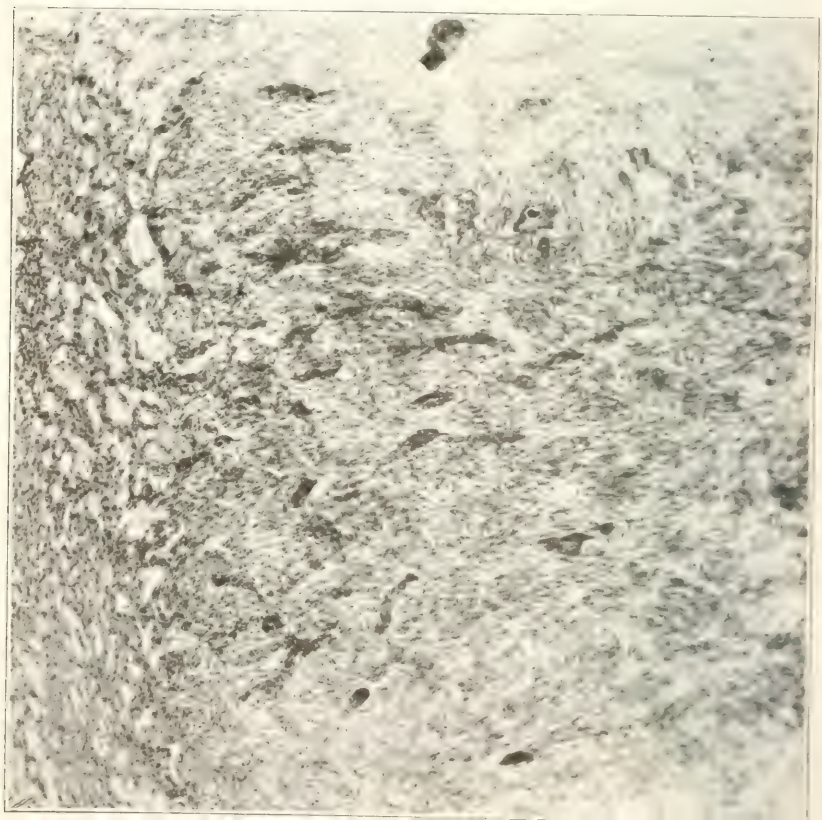


Fig. 3.—Microscopic section of the large spindle cell sarcoma of the stomach.



Prostate Gland.—There was no marked gross pathologic change.

Testicles.—They were small. The surfaces made by sectioning were smooth and yellow. The tubules were easily removed.

Aorta.—The abdominal and thoracic aortae were normal in caliber and thickness. There were a few small yellow plaques about from 2 to 4 mm. in diameter in the abdominal aorta.

Lymph Nodes.—The retroperitoneal lymph nodes were unchanged. The organs of the neck and brain were not examined.

Stomach.—The tumor was located at the cardiac portion, and was 13 cm. wide and 10.3 cm. long. It was 5 cm. thick near the center, firm, hard, grayish white and cut with resistance. Most of the tumor mass was along the greater curvature. The center of the tumor was markedly ulcerated, soft and irregular. The surfaces made by sectioning had a fibrous appearance. The outer surface of the stomach in the region of the tumor was the size of an ostrich egg and was markedly adherent to the diaphragmatic surface. The stomach weighed 1,680 gm. It was larger than normal. From the lower edge of the tumor to the pylorus it was 18.2 cm. long and averaged 8.5 cm. wide. The rugae were distinct and the surfaces were mammillated.

Liver.—It weighed 1,055 gm., and was studded with huge nodules, the largest measuring 2 by 1.8 cm. in its largest diameter, and the smallest 1 by 2 mm. These nodules were umbilicated, grayish yellow, hard and cut with resistance. On the surfaces made by sectioning there were a few small, transparent regions surrounded by darker yellow zones. The centers were often necrotic. These surfaces were grayish yellow and had a fibrous-like arrangement of the tissue. The lower surface of the inferior lobe was not so greatly involved as the upper portion, but the surfaces made by sectioning had as much involvement.

Anatomic Diagnosis.—Primary neoplasm of the cardiac portion of the stomach; metastatic neoplasm of the liver; chronic interstitial nephritis; emphysema of the lungs; general arteriosclerosis.

Microscopic Pathology.—The cells in the tumor were arranged in bundles and had replaced the normal tissue. The bundles and cells were closely packed together. The cells were spindle shaped and the majority were large, hyperchromatic and irregular. The nuclei contained much chromatin and there were nucleoli in 80 per cent. of the cells.

Liver.—The nodules contained cells closely resembling those described in the foregoing paragraph. Normal liver tissue surrounded these nodules, but the liver cells immediately surrounding the tumors were pyknotic and loosely arranged. On the outer edge of the nodules were islands of pyknotic liver cells.

Microscopic Diagnosis.—Large spindle-cell sarcoma of the stomach, with metastatic sarcoma of the liver.

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THE PHYSIOLOGICAL MAXIMUM RATE OF THE HEART

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Hooker (1), makes the statement that after bilateral vagotomy, the heart has a physiological maximum rate beyond which no further increase is possible. Stimulation of an afferent nerve fails, under these conditions, to give any further acceleration of the heart. Recently I made a similar statement (2), and the experiments reported here were begun with the idea of testing the validity of this statement by further evidence. In order to do this, I decided to use some stimulus other than that coming in over an afferent nerve. Adrenalin[1] was chosen, since by its intravenous injection a powerful stimulant would be carried to the heart in the blood stream.

There is strong presumptive evidence, at least, at hand now that cardiac acceleration above a certain autochthonous rate in an animal does not come about as the result of inhibition of the inhibitory mechanism; consequently section of the vagi does not in any way interfere with any increase in heart rate which we might afterwards obtain. Hunt (3) recorded the fact that he could never obtain an acceleration of the heart after double vagotomy as proof that cardiac acceleration must occur as the result of inhibition of these nerves, but he did not take into consideration the possibility of a physiological maximum heart rate after section of the vagi. Stewart (4) and his collaborators noted that the usual changes in heart rate during cerebral anemia were never so prominent after division of the vagi. This fact is now being studied in greater detail by Dr. Helen C. Coombs.[2]

The injection of adrenalin under ordinary circumstances results in a rise of blood pressure and a markedly accelerated heart, but sometimes the heart may become inhibited reflexly through the vagus mechanism if the vagi are intact. If the vagi are intact, the heart is not beating at its physiological maximum rate, if we assume that the maximum rate is reached only after double vagotomy, and consequently we would expect the possibility of an increase in rate when the vagi are intact. Stimulation of many sensory nerves gives the same results as adrenalin when the vagi are intact. The object of these experiments was to find out if there

[1] Suggested by Prof. F. H. Pike.

[2] Personal communication.

is any further increase in the rate of the heart on injection of adrenalin after the vagi are sectioned. If there is, then we are not justified in concluding that the heart has reached its physiological maximum rate after double vagotomy. On the other hand, if there is no marked increase in the heart rate, we are justified in concluding that the heart has reached its physiological maximum rate, beyond which it can not be any further increased after bilateral vagotomy.

We are justified in using adrenalin for these experiments because it is known that adrenalin acts directly on the heart. According to Stewart (5), the heart is directly stimulated by adrenalin. Levy (6) and Gasser and Meek (7) have also shown that a denervated heart will respond by a quickened beat to extremely small concentrations of adrenalin injected intravenously. The increase in rate may be as much as 28 beats per minute. Elliot (8) has shown that the action of adrenalin on any part of the body is practically identical with that of excitation of the sympathetic nerve supply to that part.

The action of adrenalin on the isolated heart is best shown on the perfused heart or in the heart-lung preparation. On adding this substance to the blood passing through such a preparation an effect is at once produced. This action is, like that of the sympathetic nerves, accelerator and augmentor. Through its influence, the rhythm of the heart is markedly increased in the dog to about 240 per minute (9).

Cannon (10) has employed the completely denervated heart as an indicator of adrenal secretion. Since this organ is no longer under the control of nervous impulses, it is acted upon only by agencies acting through the circulation. It is highly sensitive to circulating adrenalin. Afferent stimulation causes the heart, disconnected from the central nervous system, to increase to a marked degree its rate of beat. In the published reports the increments ranged from 24 to 52 beats per minute. After the adrenals were removed, there was no increase of rate or only a slight increase.

In another publication (11) the same author points out that the striking cardiac acceleration which follows afferent stimulation when the adrenals are the only responsive abdominal viscera, and the absence of this reflex acceleration when thereafter the adrenals are removed, can be explained only by the action of secreted adrenalin reflexly increased and carried to the heart in the circulation.

He further proves in the same paper that the denervated heart in the "reduced" animal is a means of assaying adrenalin since repeated equal doses injected intravenously at a uniform rate repeatedly produced in a given animal the same or nearly the same maximal increases of heart beat and if the injections differed in rate, the increases likewise differed in degree.

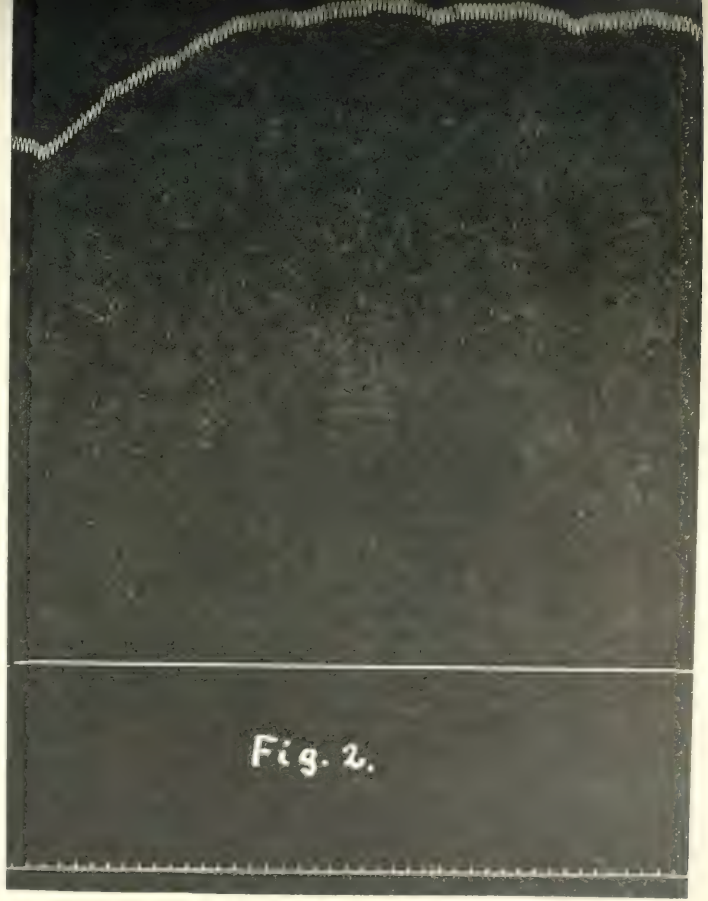

Fig. 1. Heart rate and blood pressure after bilateral vagotomy.
Fig. 2. Heart rate and blood pressure after double vagotomy followed by injection of adrenalin.

From these experimental facts it would seem that we are justified in using adrenalin in experiments in which we wish to determine whether any further acceleration of the heart rate is possible after bilateral vagotomy.

Experimental procedure. All the experiments were done on cats. Blood pressure and heart rate were recorded by means of a mercury manometer connected to the left carotid artery. Tracings were made on a Hürthle kymograph. The time was recorded in seconds by means of a Jacquet chronograph. A signal magnet served as the base line for blood pressure and also indicated the time of injection of adrenalin or of any other experimental procedure. Adrenalin was injected by means of a hypodermic needle which was plunged into the femoral vein. The heart rate was later computed in 10-second intervals.

Effect of injection of adrenalin on heart rate after double vagotomy. After a normal tracing of blood pressure and heart rate was taken, both vagi were sectioned. The blood pressure and heart rate increased, and remained at the higher level. A small amount of adrenalin was then injected into the femoral vein. Immediately the pressure rose, in some cases to double the original pressure. On computing the heart rate, however, it was found that there was no change in the rate after the injection of adrenalin from what it was after double vagotomy.

In a previous publication (loc. cit.) I have also shown that there is no increase in the rate of the heart on stimulation of a sensory nerve after double vagotomy. Further experiments of this nature were also done here and the same results were obtained.

It would seem from the results obtained here that Hooker's statement in regard to the physiological maximum rate of the heart is correct and his view is further confirmed by these observations.

	BLOOD PRESSURE	HEART RATE IN 10 SECOND PERIODS
After double vagotomy	140	35
After injection of adrenalin	180	35

SUMMARY

Twenty experiments were done on cats to determine if the heart rate could be further increased after section of the vagi, by injection of adrenalin. A tracing of blood pressure and heart rate after double vagotomy and before injection of adrenalin, and another tracing of the blood pressure and heart rate after adrenalin was injected, are given. These two tracings are representative of a great many which were obtained.

The results obtained seem to show that the heart beats at its physiological maximum after double vagotomy and that no further increase in rate

can be obtained either by injection of adrenalin or by the stimulation of a sensory nerve. The recognition of this fact would help to clear up much of the confusion in the literature regarding cardiac acceleration.

CONCLUSION

After both vagi have been sectioned, the rate of the heart is at its physiological maximum and it can not be increased any further by stimulation of a sensory nerve or by the injection of adrenalin. Hooker's statement is further confirmed by these experiments.

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THE EINTHOVEN STRING GALVANOMETER. A THEORETICAL AND EXPERIMENTAL STUDY*

By Horatio B. Williams

Introduction

More than twenty years have elapsed since Einthoven described his string galvanometer (1) which has come to be widely used in physiological laboratories as well as in hospitals and clinics. Among those who are familiar with this instrument the question has often arisen why it has never come into general use among physicists and engineers. There are probably a number of reasons. Einthoven's original instrument was designed to meet his own laboratory requirements without regard to the problem of future duplication and it did not prove attractive to instrument makers on account of the cost of production. When the importance of the work being carried on in the Leyden laboratory created a demand for string galvanometers in other institutions, instrument makers attempted to design models of less expensive type. The designers of these instruments were at a disadvantage in having had no experience in the actual use of a string galvanometer and none of these early models approached Einthoven's original instrument in convenience, sensitivity or precision. The writer still has vivid recollections of his astonishment at seeing the performance of the Leyden instrument after having worked for two years with one of the early commercial types. These early models were by no means inexpensive and still cheaper designs were put out, sometimes, unfortunately, with extravagant claims to the effect that they were fully equivalent to the Leyden instrument. On account of their low price some of these found their way early into physical laboratories and being entirely unsuited for serious work they tended to discredit the string galvanometer and create an impression that it is a troublesome instrument to handle. This was particularly regrettable as it has probably prevented the inception of much useful investigation.

Some ten years ago fortuitous circumstances led the writer to undertake the design of a string galvanometer intended to approximate the good qualities and performance of the original design of Einthoven without presenting as much difficulty from the standpoint of manufacture. The success which attended this effort has led to the design of two other types one of which is still undergoing development. These will be fully described in a subsequent section of this series of papers. In the course of this work much data has been amassed regarding the physical constants of these and other models of the string galvanometer and some new methods of determining important constants have been developed. No systematic study of the constants of any string galvanometer has been published so far as the writer is aware except the very complete data regarding the Leyden instrument communicated by Einthoven (2, 3). It seems desirable therefore, to make some of this material available in connection with the description of the new designs above referred to, especially as these data relate to instruments now widely used. One of the Editors of this Journal suggested that it might be wise to extend the scope of the paper so as to include the mathematical theory, general principles of design and construction and a discussion of applications other than physiological for which the string galvanometer is especially suitable. The theory of the instrument has been treated by several authors, (2, 3, 4, 5, 6, 7, 8) and it was the writer's

*This paper deals with the theory. A second paper will follow at an early date.

original intention not to discuss it. It has seemed best after consideration to do so partly because [illegible] the implication of Crehore's paper all the material of the theory have been printed in foreign languages and some in journals not readily accessible to [illegible] physicists. Einthoven's original discussion of the theory assumes that the string galvanometer may be regarded as a system with but one degree of freedom. An analysis based on this assumption actually gives a sufficiently close approximation to serve as a basis for the use of the instrument, but leaves doubts regarding certain points until these have been disposed of by a more general analysis. Hertz (4) attempted a general analysis but the usefulness of his work was limited by his apparent unfamiliarity with the actual instrument. Fahr (6) sets up the partial differential equation, but avoids it and proceeds to treat the problem by means of an ordinary differential equation, which again limits the generality of the result to a single degree of freedom. His paper is excellent and very practical and it is unfortunate that, although written by an American, it was published in a German journal which limits its accessibility. The first really general treatment of the theory is that of Crehore, (7) but his paper seems to have proved difficult readers to many [illegible] to understand form and the use of the mean of [illegible] to obtain a [illegible] to make comparisons. Crehore's conditions have been [illegible] to [illegible] to the average reader the manner in which they might be applied to a concrete problem and he has raised [illegible] regarding the effect of the partial modes of the string's vibration on the accuracy of the records which seems to require further investigation, particularly as the [illegible] field of an uniform magnetic field, differs measurably from the actual working condition. His discussion of the partials seems to have raised doubts as to the availability of the instrument for precise analysis of wave form and similar applications without supplying data for a decision. There has recently come to the writer's attention a paper by Pochettino (9) which the reader who is familiar with Italian will do well to consult. The paper [illegible] for the sake of simplicity treats the problem on the basis of one degree of freedom, first, however, indicating the lines of development of the general theory.

It seems desirable in view of what has been mentioned to attempt to treat this subject once more with sufficient generality to answer the questions [illegible] raised but followed by the approximate theory. The writer is an experimentalist and makes no pretense to proficiency in the application of mathematical analysis. For this reason he has hopes that the paper may not present serious analytical difficulties to other experimentalists whose mathematical attainments may be of the same general order of mediocrity as his own. In order to make more certain of meeting the needs of the class of readers for whom [illegible] he proposes to include more of the steps of analysis than is usually done in theoretical papers. In order not to divert attention from the main issues, these intermediate steps [illegible] developed in an appendix to which reference will be made by small letters, a, b, etc. Citations of related matter in standard treatises on mathematics [illegible] will be made. The method of applying the results to concrete problems will be indicated and illustrative examples will present themselves naturally [illegible] of the actual instrument.

It is a matter of some difficulty to make proper acknowledgment of the sources of mathematical methods used. These have come to be more or less common property. The following well known works have been freely consulted: Thomson and Tait's Natural Philosophy, Rayleigh's Theory of Sound, *I*, chapters III-VI inclusive, Byerly's Fourier's Series and Spherical Harmonics, Forsyth's Treatise on Differential Equations and Williamson's Integral Calculus. In particular the complementary function of equation (8) has been taken with a slight transformation from Professor Byerly's book, p. 115. The solution of (7) for small values of the damping is well known as it is one of the important equations in the theory of sound. The solutions for critical and more than critical damping I have not seen in the literature, though it would be remarkable if they have not been given. The solutions for (15) and (16) are original. I am indebted to Professor Laws [illegible] valuable suggestions gleaned from his discussion of the theory of moving coil and moving [illegible] instruments to be found

on Electrical Measurements. My obligation to previous writers on the theory of the string galvanometer is obvious, but particular mention should be made of Dr. Crehore's paper.

PART I. THE MATHEMATICAL THEORY

1. STEADY CONTINUOUS CURRENT, EQUILIBRIUM.

For the purpose of this part of the discussion, the string galvanometer may be described as consisting of a fixed magnetic field of considerable strength, 20,000 gauss or more, uniform in the direction of the string's motion and fairly so in the direction of its length. In this field is stretched a very light conductor, usually a quartz fiber coated with a thin layer of silver or gold. The usual diameter of these fibers is from .001 to .0025 mm. The motion of the fiber is magnified by means of a microscope introduced through a perforation in the middle of the polepiece, the opposite polepiece being likewise perforated for a similar microscope used to illuminate the string. The magnified shadow may be observed directly, or projected, and its motion when too rapid for visual observation, may be recorded photographically after the manner familiar to users of the oscillograph. Micrometric adjustment is provided for altering the tension of the string and in this manner the period and sensitivity of the instrument may be altered over a wide range.

We shall first develop the equations which apply when a steady continuous current has flowed through the string long enough so that equilibrium has been established. In this section of the paper we may assume that the field is perfectly uniform. The actual distribution of the field and its effect on the string's motion and also a method for determination of the uniform effective field in which the steady deflection for continuous current will be the same as in the actual field will be considered later.

Let H be the strength of the effective field in gauss,
I, the steady continuous current in *cgs* units,
U, the deflection of the middle point of the string in centimeters,
l, the length of the string in centimeters,
m, the linear density or mass per unit length of the string in grams per cm.
τ_0, the free period of the string in seconds, without damping,
T, the tension on the string in dynes,
r, the radius of curvature of the string at any point, in centimeters.

The force acting on any elementary length, dl, of the string, is $HIdl$. Under the condition of equilibrium, this is balanced by the elastic restoring force due to the tension on the string. The tension is assumed to be so great that the slight increase in it due to the deflection, which is assumed small, is negligible in comparison. We proceed to find an expression for this restoring force.

In Fig. 1, T_1 and T_2 represent the tensions acting at the ends of an infinitesimal arc, dl, of the string, and may be regarded as prolongations of the tangent lines at the ends of the arc. The elastic restoring force is evidently numerically equal to the normal com-

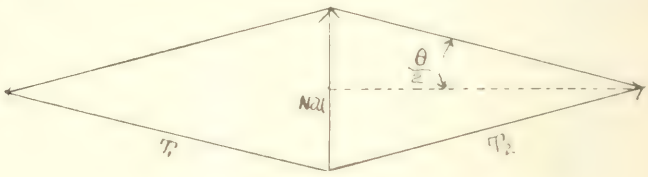

FIG. 1.

ponent of these tensions, Ndl. Take T as the arithmetical mean of the two tensions, T_1 and T_2, which in no case can differ greatly since the arc is infinitesimal. θ is the angle through which the tangent line turns in passing from one end of the arc to the other, this angle differing by an infinitesimal amount from zero. Then $\frac{1}{2}\frac{Ndl}{T} = \sin\frac{\theta}{2}$, or $Ndl = 2T \sin\frac{\theta}{2}$. Since θ is an infinitesimal angle, $\sin\frac{\theta}{2}$ is equal to $\frac{\theta}{2}$ and $Ndl = T\theta$. By the definition of an angle in terms of the arc subtended and the radius of curvature, $\theta = dl/r$, whence,

$$Ndl = \frac{T}{r}dl \qquad (1)$$

(1) may be stated in words, thus: the normal component of the tension over any elementary length of the string is numerically equal to the average tension throughout the elementary length divided by the radius of curvature at this element. (Appendix a).

When no current is passing through the string, the tension is uniform throughout its length. If the impressed forces have tangential components, then in the deflected position these tangential components will act along the string to produce variations of tension. In the uniform field in which we assume the string to be, the forces act everywhere perpendicular to the lines of force and perpendicular to the string.

The curvature will be in a plane at right angles to the lines of the fixed field. Initially all the forces acting on the string are parallel to each other since the undeflected string is straight. So soon as deflection begins to take place a new disposition of things arises. If the string were like a chain or cable, stretched horizontally and acted upon by gravity, the forces would remain parallel to each other as deflection takes place. The force acting between a flexible conductor carrying current and a fixed homogeneous field at right angles to it is everywhere perpendicular to *each elementary length of the conductor*. This is true in deflected as well as in undeflected positions. Since the forces are everywhere at right angles to each element of length of the string, it follows that the tangential components of force are everywhere zero and the tension, T, is constant throughout the string in deflected as well as in undeflected positions. From (1) we have that the normal component of tension, $Ndl = \frac{T}{r} dl$, and this is equal to the impressed force, $HIdl$, over the same element of length since the forces are in equilibrium. Since (1) the current through the string has everywhere the constant value I, (2) the field has everywhere the constant value H and (3) we have shown that the tension is uniform, we may dispense with the differential notation and write simply, $\frac{T}{r} = HI$ per centimeter length. Hence we see that

$$\frac{1}{r} = \frac{HI}{T} \qquad (2)$$

That is, the reciprocal of the radius of curvature is a constant. The reciprocal of the radius of curvature is a measure of the curvature and the curve of constant curvature, (radius of constant length), is a circle.

The form of the string when deflected by constant current in an uniform field is therefore a circular arc. (Appendix, b).

In Figure 2, let AC be the undeflected string of length $l = AC$ and let ABC be the circular arc into which it is deflected by the electromagnetic forces when it is traversed by the current, I, in the fixed field, H. The deflection of the middle point, B, from the position it occupies when the string is a straight line, we shall call U. O is the center of the circular arc and OB, OA, are radii, equal to r.

From the geometry of the figure we deduce without difficulty, neglecting the square of the small quantity, U, that

$$r = \frac{l^2}{8U} \tag{3}$$

(See appendix c).

Equation (2) may be written, $T = HIr$ and on substituting in this the value of r, just obtained, we have,

$$T = \frac{HIl^2}{8U} \tag{4}$$

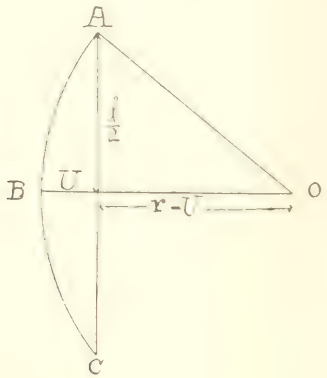

FIG. 2

The equation connecting the length, linear density, tension and free undamped period of a vibrating stretched string is, $\tau_0 = 2l\sqrt{\frac{m}{T}}$. We shall derive this equation later in connection with the solution of the equations of motion of the string. For the present we shall assume it. (It can be found in almost any elementary discussion of acoustics). From it we readily obtain,

$$T = \frac{4l^2 m}{\tau_0^2} \tag{5}$$

Equating the right hand members of (4) and (5), l^2 drops out and we obtain,

$$H = \frac{32\, m\, U}{I\tau_0^2} \tag{6}$$

It is obvious that this equation may be written in various forms in order to get any one of the quantities involved in terms of the others. As it stands, it indicates a method of determining the field strength experimentally. This method will later be described in detail and for the present it may be assumed that all of the

quantities in (6) are readily determinable with a good degree of accuracy.

2. THE EQUATIONS OF MOTION

The partial differential equation of motion of a stretched string, subject to the action of a periodic impressed force and to damping forces of frictional or other nature which may be treated as simply proportional to the velocity of the string's motion, is, (Appendix d),

$$\frac{\partial^2 u}{\partial t^2} + \frac{k}{m}\frac{\partial u}{\partial t} - a^2\frac{\partial^2 u}{\partial x^2} = \sum_{n=1}^{n=\infty}\sum_{s=1}^{s=\infty}\frac{HI_n}{m}\cos n\omega t \cdot \sin\frac{s\pi x}{l} \qquad (7)$$

In this equation $a^2 = \frac{T}{m}$ and $\omega = 2\pi f$, f, being the fundamental frequency of the periodic impressed force; t, is elapsed time, u, the ordinate, and x, the corresponding abscissa of any point on the string, the origin being taken at one end of the string and the zero position of the string as coincident with the X-axis. I_n is the maximum amplitude of any harmonic component of the current through the string, n, being the order of any harmonic and equal to unity for the fundamental frequency. The length of the string, as before is, l, k, is the coefficient of damping, and includes all damping from whatever cause. s, is an integer which may be interpreted as representing the order of any partial mode of the string's vibration. The double sign of summation indicates that a doubly infinite series of equations of this form must be written to express the motion completely, corresponding to the infinite number of values which n and s can separately assume, the second member of the equation representing any periodic force acting at every point of the string. The factor, $\sin\frac{s\pi x}{l}$, indicates that the shape of the string will be represented by a series of sine curves such that for $x = 0$ and $x = l$, the deflection will always be zero.

The cosine factor in the second member indicates that we may assume the periodic impressed force to be made up of an infinity of simple periodic terms to each of which there corresponds a particular value of I_n. We might have represented this force by a series of sine terms, but it may assist in avoiding confusion with the terms expressing the shape of the string to use a cosine series for the force. The shape of the string could also have been expressed in cosine terms, but in that case the origin would have to be taken at the middle point of the string instead of at the end. The results would always be the same. In general a Fourier series is made up

of *both* sine and cosine terms. The string galvanometer like other electromagnetic devices can have no even harmonics developed, on account of the manner of application of the force. This is so well and generally understood that further discussion of it seems superfluous. For the sake of simplicity in the form of the equations it has been assumed that the impressed force contains no even harmonics as would likely be the case if it is produced by electromagnetic devices. Since the origin of time may be taken anywhere we are at liberty to represent the force by either sine or cosine terms at will.

The solution of (7) assumes three distinct forms according to the relative values of the restoring and damping forces. Regarding the restoring force as constant, the damping may be,

(a) insufficient to prevent oscillations,
(b) just sufficient to prevent oscillations,
(c) more than sufficient to prevent oscillations.

For condition (a), the solution is, (Appendix d),

$$u = \frac{2}{l} e^{-\frac{kt}{2m}} \sum_{s=1}^{s=\infty} \left[\sqrt{\frac{1}{1 - \frac{l^2 k^2}{4m^2 s^2 \pi^2 a^2}}} \cos\left(t \sqrt{\frac{s^2\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}} - \tan^{-1} \frac{k}{2m \sqrt{\frac{s^2\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} \right) \sin\frac{s\pi x}{l} \int_0^l f(\lambda) \sin\frac{s\pi\lambda}{l} d\lambda \right]$$
$$+ \sum_{n=1}^{n=\infty} \sum_{s=1}^{s=\infty} \frac{HI}{\sqrt{k^2 n^2 \omega^2 + m^2 \left(\frac{s^2\pi^2 a^2}{l^2} - n^2\omega^2 \right)^2}} \cos\left(n\omega t - \tan^{-1} \frac{kr\omega}{m\left(\frac{s^2\pi^2 a^2}{l^2} - n^2\omega^2 \right)} \right) \sin\frac{s\pi x}{l} \quad (8)$$

The first term of this solution, the complementary function, defines a transient motion dependent upon the properties of the moving system solely. The second term, or particular integral defines the forced motion which the system will be constrained to execute when the particular force represented by the second member of (7) acts, and after enough time has elapsed to permit the transient motion to decay to an insignificant amount. For a short time after the periodic force begins to act the motion will be defined by both terms of the solution. The forced motion depends upon the nature of the im-

pressed force and *also* upon the character of the moving system. The assumption of a periodic impressed force is perfectly general provided we consider that the period may be infinite, that is the force may be due to a steady continuous current, or the force may be zero, which does not necessarily imply that nothing will happen, but rather that a force is removed entirely while the string is in some deflected position. The solution for the latter condition is simply the complementary function and in the case of less than critical damping, this term contains trigonometric functions which indicate that the deflection subsides in a series of oscillations. $e^{-\frac{kt}{2m}}$ is a factor of the entire complementary function and expresses the law of decay of the motion. The letter e, is the base of the natural system of logarithms and the negative sign shows the expression to be a fraction which diminishes without limit as time elapses.

The period of the natural vibrations of the string under damping k, is for the partial of order s, $\frac{2\pi}{\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}}$. The fundamental damped period is $\tau_k=\frac{2\pi}{\sqrt{\frac{\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}}$ derived from the above by putting $s=1$.

It is evident that one of the effects of damping is to increase the period. Since the damping of the fundamental in the string galvanometer is always large, this effect will be marked. Since s^2 is a factor of the restoring force term and not of the damping term, the former increases with the order of the partial while the latter does not. As a result the partials will not be exact multiples of the fundamental and so, strictly speaking, not harmonics. We shall refer to them throughout as "partials" as this will tend to prevent confusion of them with the harmonics of an impressed force.

The period without damping for the partial of order s, is $\frac{\tau_0}{s}=\frac{2\pi}{\sqrt{\frac{s^2\pi^2a^2}{l^2}}}=\frac{2l}{sa}$ and the fundamental period without damping is, $\tau_0=\frac{2l}{a}$, or, putting for a, its value $\sqrt{\frac{T}{m}}$, $\tau_0=2l\sqrt{\frac{m}{T}}$ which is the equation from which equation (5) was derived.

The term, $\tan^{-1} \frac{k}{2m\sqrt{\frac{s^2\pi^2a^2}{l^2} - \frac{k^2}{4m^2}}}$ in the complementary function, is known as the phase angle term. Its presence shows that the motion of the string when executing natural vibrations lags behind the forces due to tension and inertia which produce the motion. In this term also s is a factor of the restoring force and not of the damping force so that the various partial modes of vibration will not remain in phase with each other as the motion dies away. There is also a phase angle term in the particular integral which is of more practical importance in that it gives a measure of the amount by which the motion of the string will lag behind the impressed forces when it is performing forced vibrations. The expression for the tangent of this angle shows that the amount of lag will be different for each different order of harmonics in the impressed force. Many writers on the theory of recording instruments have pointed out the significance of this term, which is common to the equations of motion of all such instruments, as showing that the record can never be a faithful graph of the impressed forces. Just how serious a matter this really is can be better understood by making some numerical computations but as the string galvanometer is best used for measurements of precision under condition (b), or critical damping, this discussion will be deferred until the solution of the differential equation for that condition has been developed.

The nature of the transient motion defined by the complementary function depends on the shape which the string assumes when deflected by the electromagnetic forces. We have shown in the first section that in an uniform field this form will be a circular arc. The factor of the solution which takes account of the form of the string is, $\frac{2}{l} \sin \frac{s\pi x}{l} \int_0^l f(\lambda) \sin \frac{s\pi\lambda}{l} d\lambda$, which for brevity we may designate, $F(x)$. $F(x)$ is the general expression for a Fourier series, (containing in this case sine terms only as before explained), and the definite integral when the proper function is substituted for $f(\lambda)$ and the integration performed, gives the form of the coefficients of the series, which can then be immediately written down, giving s all odd integral values.[1] In the definite integral which occurs in $F(x)$, λ is written instead of x in order to emphasize the fact that

[1] Probably the best elementary discussion of the development and use of Fourier series is to be found in Byerly's Elementary Treastise on Fourier's Series, etc. Ginn and Co. Boston, 1893.

it is a function of the limits of integration and thus prevent confusion with x used elsewhere as an independent variable.

If the field distribution is not such as to make the shape of the string a circular arc the equation will still give the solution provided the equation of the string can be expressed in some recognized analytical form and provided the integration can be performed. We shall have occasion to consider solutions for shapes other than the circular arc.

Although this solution in the form of a double infinity of equations might seem to be an academic performance impossible of application to particular problems, such is by no means the case.

It is already evident that much information of a general nature can be obtained from consideration of the form of these solutions, but on account of the usually rapid convergence of the series which arise from them, a relatively small number of terms may afford an entirely satisfactory approximation to the solution of a numerical problem.

By far the most important mode of vibration of the string is the fundamental and for this mode the solution can be reduced to a very simple form in which the quantities involved are all of such a nature as readily to be determinable. Remembering that for the fundamental mode, $s = 1$, we can readily derive from the expressions already given for τ_0 and τ_k (Appendix e),

$$\frac{k}{2m} = 2\pi \sqrt{\frac{1}{\tau_0^2} - \frac{1}{\tau_k^2}} \quad \text{so that } e^{\frac{k}{2m}t} = e^{-\frac{t}{\tau_0}} \sqrt{\frac{1}{\tau_0^2} - \frac{1}{\tau_k^2}}$$

Also,

$$\sqrt{\frac{1}{1 - \frac{l^2 k^2}{4\pi^2 m^2 a^2}}} = \sqrt{\frac{\frac{\pi^2 a^2}{l^2}}{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} = \sqrt{\frac{\tau_0^2}{\tau_0^2 - \tau_k^2}}$$

In the cosine term,

$$\sqrt{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}\, t = \frac{2\pi}{\tau_k} t$$

In the phase angle term,

$$\frac{k}{2m \sqrt{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} = \frac{k}{2m} \times \frac{1}{\sqrt{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} = \frac{\tau_k}{2\pi} \sqrt{\frac{\tau_0^2}{\tau_0^2 - \tau_k^2}} = \sqrt{\frac{\tau_k^2}{\tau_0^2 - \tau_k^2}}.$$

On substituting these values, the complementary function becomes ($s=1$)

$$u=\frac{2}{l}e^{-2\pi t\sqrt{\frac{1}{\tau_0^2}-\frac{1}{\tau_k^2}}}\left[\frac{\tau_k}{\tau_0}\cos\left(\frac{2\pi t}{\tau_k}-\tan^{-1}\sqrt{\frac{\tau_k^2}{\tau_0^2}-1}\right)\sin\frac{\pi x}{l}\int_0^l f(\lambda)\sin\frac{\pi\lambda}{l}d\lambda\right] \quad (9)$$

In nearly all of the applications of the string galvanometer, it is desirable that natural vibrations of the string shall not appear to complicate the record. Condition (b), critical damping, or as it is often called, the "just aperiodic condition," is the condition of choice. The string galvanometer lends itself nicely to use in this condition as it can be readily brought to exact aperiodicity by adjustment of the tension. Ordinary moving coil and moving magnet galvanometers are usually referred to as "critically damped" when the damping is such that the motion is *slightly* oscillatory. Because of this common usage of the term I wish particularly to emphasize that whenever the term "critical damping" is used in this paper it implies a very precise adjustment and that no oscillations occur. The user of the string galvanometer can determine the adjustment which gives exactly critical damping with the greatest nicety either by stroboscopic means[2] or by making several photographic records. Since the determination of the important constant, τ_0, depends on the precision of this adjustment, it is desirable that the matter be entirely clear.

The criterion for critical damping is that the cosine term of the complementary function of (8) shall just vanish. We readily see that this will occur when $\frac{k}{2m}=\frac{s\pi a}{l}$. This is the criterion for critical damping of the sth. partial of the string's motion. Although it is critical damping of the fundamental mode in which we are mainly interested, still for the sake of generality we may retain s until the solution is complete and then obtain the equation for the critically damped fundamental by merely putting s equal to unity. When $\frac{k}{2m}=\frac{s\pi a}{l}$, the auxiliary equation, (Appendix f), has two iden-

[2] See A. Forbes and D. W. Mann. This Journal Vol. 8, p. 807.

tical roots, $\beta_1=\beta_2=\beta=-\frac{k}{2m}$ and we have as the complementary function,

$$u=e^{\beta t}\left\{\frac{2}{l}\sum_{s=1}^{s=\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda\right.$$
$$\left.-\frac{2\beta t}{l}\sum_{-1}^{-\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda\right\} \quad (10)$$

This solution satisfies the differential equation, is zero when x is zero and when x equals l. Differentiating it with respect to t, we find,

$$\frac{\partial u}{\partial t}=\beta e^{\beta t}\left\{\frac{2}{l}\sum_{s=1}^{s=\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda-\frac{2\beta t}{l}\sum_{-1}^{-\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda\right\}$$
$$-\frac{2\beta}{l}e^{\beta t}\sum_{-1}^{-\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda.$$

When $t=0$ the first and third terms of the derivative cancel each other and the second is zero, so that the derivative vanishes as it should, since the first derivative represents the velocity of the string string and at the instant that it is let go from a position of equilibrium it has no velocity. All of the boundary conditions are therefore satisfied.

To (10) must be added the particular integral which defines the forced motion due to a periodic impressed force under the condition of critical damping. This may be derived from the particular integral in (8) by putting $\frac{k}{2m}=\frac{s\pi a}{l}$, from which we have, $k=\frac{2s\pi am}{l}$. Substituting this value of k and reducing, (Appendix, g), we have as the particular integral for critical damping of the sth. partial mode of the string's motion,

$$u=\sum_{n=1}^{n=\infty}\sum_{s=1}^{s=\infty}\frac{HI_n l^2}{s^2\pi^2T+mn^2\omega^2l^2}\cos\left(n\omega t-\tan^{-1}\frac{2s\pi an\omega}{l\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}\right)\sin\frac{s\pi x}{l} \quad (11)$$

If k and m were both zero, the amplitude factor in (11) would reduce to,

$$\frac{HI_n l^2}{s^2\pi^2T} \quad (12)$$

See Appendix A, Part 2

The phase angle would be zero and the forced vibrations would be directly proportional to the impressed force and inversely proportional to the tension on the string. These are the properties of a perfect instrument and while they cannot be realized, their mathematical formulation is of interest in that it enables us to determine the extent of the distortion of the amplitudes which inertia and damping introduce in an actual instrument. The occurrence of the quantity s^2 in (12) requires comment. In connection with a real string it would indicate that the factor in which it occurs expresses the amplitude of the sth. partial. For a massless string this has no meaning, but we can assign it a meaning by allowing it to represent the amplitude of a massless string under tension T and of length $\frac{l}{s}$, equal to the distance between the nodes of the sth. partial of a string of mass, m, and length, l, under tension, T. If $\underline{U}_s$ represent the amplitude of the motion of the massless string and $\underline{U}_m$ that of the one of mass, m, with damping, k, we may determine the ratio of these amplitudes in order to obtain a measure of the amplitude distortion for any impressed frequency.

$$\frac{\underline{U}_s}{\underline{U}_m} = \frac{HI_s l^2}{s^2\pi^2 T + mn^2\omega^2 l^2} \Big/ \frac{s^2\pi^2 T}{HI_s l^2} = \frac{1}{1+\frac{n^2\omega^2 l^2}{s^2\pi^2 a^2}}, \text{ remembering that } T/m = a^2.$$

Putting $\omega = 2\pi f$, where f is the fundamental frequency of the impressed force, and bearing in mind that $\frac{l^2}{a^2} = \frac{\tau_0^2}{4}$,

$$\frac{U_s}{\underline{U}_m} = \frac{1}{1+\frac{4\pi^2 n^2 f^2 \tau_0^2}{4 s^2 \pi^2}} = \frac{1}{1+\frac{f^2 n^2 \tau_0^2}{s^2}} \qquad (13)$$

Equation (13) holds for the sth. partial provided this is critically damped. Critical damping of a partial is not a matter of practical interest, but by putting $s = 1$, we have the ratio, for any impressed frequency, of the maximum amplitude of a perfect instrument to that of an actual instrument with critical damping of the fundamental, which is of major importance. The accompanying curve, Fig. 3, is a plot of this equation made from a large number of calculated values. The equation represents a family of such curves all of the same shape, that for $s = 1$ being the only one of importance. This curve shows in striking manner the effect on the record of a critically damped galvanometer of varying the period of the im-

pressed force for any given value of τ_n. If the free period of the string without damping, τ_0, is one tenth the period of the impressed force the amplitudes in the registered curve will be correct to 99 per cent of the theoretical value. If the impressed force contains components of period double the free period of the string, these components will be registered with an amplitude 80 per cent of their actual value. If the period of any component agrees with the free period of the string so that in the absence of heavy damping we should have resonance, under the condition of critical damping that component will be registered with an amplitude 50 per cent of its correct value relative to slower frequencies. This curve enables the

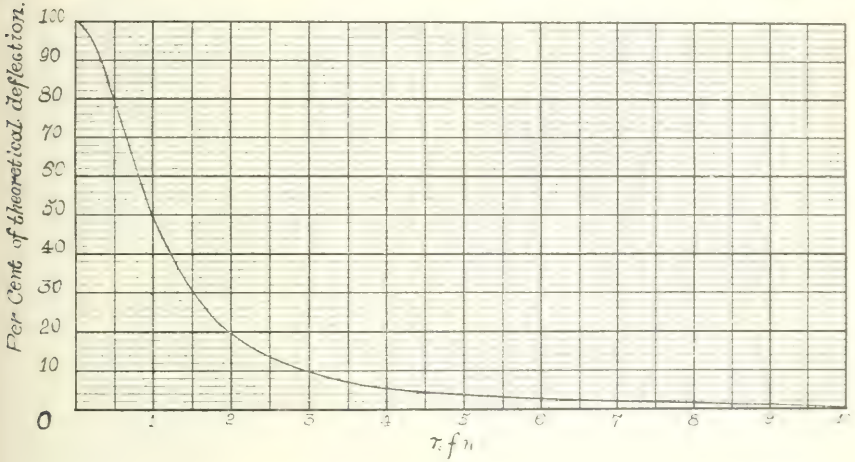

FIG. 3.

user of the galvanometer to decide at once what free period his string must have to register to any specified degree of accuracy any assigned frequency. If a curve has been registered, say for determination of wave form, and an harmonic analysis performed, a knowledge of the free period of the string will enable the user to decide whether any of the components of the wave require correction and if so how much the correction must be. The effect of the under-damped partials when the fundamental is critically damped will be discussed in a separate section devoted to general consideration of the partials.

For the condition of critical damping the phase angle term of the particular integral can also be reduced to a form which facilitates numerical computation as follows: The phase angle is

$\epsilon_s = \tan^{-1} \dfrac{kn\omega}{m\left(\dfrac{s^2\pi^2a^2}{l^2} - n^2\omega^2\right)}$. If the damping of the sth. partial is critical, $\dfrac{k}{2m} = \dfrac{s\pi a}{l}$. The undamped free period of the sth. partial we may designate $\tau_s = \dfrac{\tau_0}{s}$. Then $\dfrac{s\pi a}{l} = \dfrac{2\pi}{\tau_s}$ and $\dfrac{s^2\pi^2a^2}{l^2} = \dfrac{4\pi^2}{\tau_s^2}$ $k = \dfrac{2ms\pi a}{l} = \dfrac{4m\pi}{\tau_s}$ and $\omega = 2\pi f$ where f is the fundamental frequency of the impressed force. Then,

$$\epsilon_s = \tan^{-1} \frac{\dfrac{8m\pi^2fn}{\tau_s}}{m\left(\dfrac{4\pi^2}{\tau_s^2} - 4\pi^2f^2n^2\right)} = \frac{8\pi^2fn}{\dfrac{4\pi^2}{\tau_s} - 4\pi^2f^2n^2\tau_s} = \frac{2fn\tau_s}{1 - f^2n^2\tau_s^2}.$$

which is the expression for the tangent of the phase angle when the sth. partial critically damped. Critical damping of the partials is a matter of little practical interest, but putting $s = 1$, we have immediately the value for the tangent of the phase angle for critical damping of the fundamental,

$$\tan \epsilon = \frac{2fn\tau_0}{1 - f^2n^2\tau_0^2} \tag{14}$$

From (14) we can compute the amount of phase displacement which any component of the impressed force will suffer for any value of the fundamental free period, τ_0. It will be instructive to make this computation for a few values of n at two assumed values of τ_0. To obtain the amount of displacement in millimeters on the photographic record we proceed as follows: divide the number of degrees in the phase angle by 180 and take this fraction of the distance which the photographic surface travels in a time equal to a half cycle of the period in question. For the fundamental this will be the distance traveled in half the fundamental period, for the third harmonic the distance traveled in half the period of the third harmonic, etc.

We may consider first a galvanometer of natural period $\tau_0 = .001$ second. This is about the upper limit of the period for the large instruments using strings 13.5 cm in length. Assuming for the fundamental impressed period 100 cycles a second and a speed of the

photographic record of five meters a second, giving 25 millimeters for a half cycle of the fundamental, we have the following.

Frequency	Phase angle	Displacement
100	11.5 degrees	1.6 mm
300	33.5 degrees	1.55 mm
500	53.1 degrees	1.48 mm

Displacement of the entire record by a constant amount would be without distorting effect, it is *relative displacement* which concerns us. The relative displacement for the third harmonic would be illegible on any photographic record. The fifth harmonic would be but slightly more displaced, and reference to figure three shows that when as here the period of any component of the impressed force is double the natural period of the string, the amplitude of that component is subject to an amplitude distortion of 20 per cent. If one is obliged by circumstances to use a galvanometer under such conditions as the last mentioned, it is possible to apply corrections to both amplitude and phase provided an harmonic analysis of the record is made. It is evident however, that the amplitude distortion is much more serious than the phase displacement which for the conditions assumed is negligible.

Let us now assume for the galvanometer a period of .0001 second and a speed of ten meters per second for the travel of the record, giving 50 mm for a half cycle of the fundamental, assumed as before to be 100 cycles per second. Such short periods have not been obtainable with string galvanometers heretofore, but the writer has recently constructed an instrument which attains this period.

Frequency of impressed force	Phase angle	Displacement
100	1.166 degrees	.323 mm
300	3.5 degrees	.323 mm
500	5.73 degrees	.318 mm
700	8.0 degrees	.318 mm
900	10.33 degrees	.318 mm

The relative phase displacement is seen to be entirely negligible as far as the ninth harmonic and reference to Fig. 3 will show that the amplitude distortion of the ninth harmonic is less than one per cent. The fact that the phase angle increases rapidly with increasing order of the harmonics has often been pointed out in connection

with recording instruments, but writers, themselves, no doubt cognizant of it, have not always been explicit in calling attention to the fact that this increased angle relates to a distance on the photographic record which becomes shorter with increasing order of the harmonics and that this in great measure offsets the rapid increase in the angle. If the period of the galvanometer is so high relative to the period of the impressed forces that no correction of the amplitude is necessary, it will seldom be necessary to consider phase displacement.

In any event if the constants of the galvanometer have been determined, the equations here given may always be used to correct both phase and amplitude distortion.

Equation (10) may be put in more convenient shape for practical application by making substitutions similar to those which were used in the derivation of equation (9). Putting $s=1$, since our main interest in making this reduction lies in the fundamental mode, and writing for brevity, $F(x) = \frac{2}{l}\sum_{0}^{\infty} \sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda$,

we may write equation (10),

$$u = e^{\beta t}\left(F(x) - \beta t F(x)\right) = F(x)e^{\beta t}(1-\beta t)$$

$F(x)$ being the function which defines the shape of the string when deflected by a steady continuous current. $\beta = -\frac{k}{2m}$ and since the condition of critical damping of the fundamental is imposed, $\frac{k}{2m} = \frac{\pi a}{l}$ and since $\tau_0 = \frac{2l}{a}$, $\frac{\pi a}{l} = \frac{2\pi}{\tau_0} = \frac{k}{2m}$ so that $\beta = -\frac{2\pi}{\tau_0}$ and substituting this value of β we have,

$$u = F(x)e^{-\frac{2\pi t}{\tau_0}}\left(1 + \frac{2\pi t}{\tau_0}\right) \qquad (15)$$

Equation (15) is of great practical utility. From it we may not only determine the time required for a critically damped galvanometer to complete any assigned fraction of its steady deflection, but it also suggests a ready method of determining the important constant, τ_0.

If we deflect the galvanometer with steady current and then suddenly remove the emf and allow the string to return to zero, recording its motion photographically with a simultaneous record of the time, we can measure from the record corresponding values of elapsed

time, t, and deflection, u, reckoning $t=0$ from the point where the shadow first begins to move. The optical system of the galvanometer will not be discussed in this part of the paper, but it may be stated that satisfactory photographic records can be secured on film running at the rate of from five to ten meters a second. At these speeds accuracy of time measurement sufficient for determination of τ_0 can be attained. $F(x)$ in equation (15) we may replace by U, the maximum deflection of the middle point, for we are now interested in the fundamental mode only and, as will appear after the partial modes of the string's motion have been discussed, the partials will not affect the present measurement in any material way if the point chosen to measure t and u is the point, $t=\frac{\tau_0}{3}$. For this purpose we may then write equation (15),

$$u = U e^{-\frac{2\pi t}{\tau}} \left(1 - \frac{2\pi t}{\tau_0}\right) \qquad (15a)$$

If it were possible to determine the free period accurately by some independent means, we might from a comparison of this with the period determined as above suggested not only secure a check on the accuracy of the method, but also shed light on the question of the correspondence between the assumptions made in setting up the equation of motion and the behavior of the actual galvanometer. In all application of mathematical analysis to material phenomena it is necessary to make simplifying assumptions. We have in this instance assumed that the motion of the string may be looked upon as similar to an infinitesimal motion, that the damping is simply proportional to the first power of the velocity of the motion, that the string is perfectly flexible, etc. If any of these assumptions is far from the facts, the results of the analysis will be correspondingly affected. It is fortunately possible to check the validity of equation (15a). A description of the actual experiment will best illustrate the method. A wire of the alloy known as "duraluminum" was stretched in the galvanometer under such tension that its oscillations with air damping alone were subject to so little decrement as to make correction of the period for damping unnecessary. The period was determined from a photographic record of the oscillations. The time of 22 oscillations was measured and the time of a single oscillation determined from this was .00356 second.[3] The wire

[3] The last decimal place may be in error ±2 units.

was then shunted and a series of records made until the value of the shunt which would make the electromagnetic damping just sufficient to prevent oscillations was determined. A record of a critically damped deflection was then made on film running at five meters a second and the period determined by the following procedure. In (15a) assume $t=\frac{\tau_0}{3}$. The equation becomes,

$$u=Ue^{-\frac{2\pi}{3}}\left(1+\frac{2\pi}{3}\right)=.1237U(1+2.093)=.38U$$

In the record of movement of the shadow of the wire, U was 25 mm and $.38\times 25=9.5$ mm. This means that the deflection will have decayed from 25 mm to 9.5 mm in a time equal to $\frac{\tau_0}{3}$. To get $\frac{\tau_0}{3}$, we have only to measure elapsed time from the beginning of the motion to the point where the deflection is 9.5 mm and this was found to be .0011 second. Multiplying this by three we have as the value of τ_0 .0033,[4] which is in good agreement with that determined directly. In carrying out this experiment the latter determination was made before the record of the free vibrations was measured in order to avoid prejudice. In addition to serving as a check on the validity of the assumptions which form the basis of the analysis, this method has a further and eminently practical aspect. The fine quartz fibers which are usually employed as strings have so little inertia that when they are stretched under as great a tension as regard for their continued serviceability warrants, the air damping alone is sufficient to prevent more than one or two highly damped oscillations and with the finest strings a half oscillation is about all that can be obtained. Under these circumstances it is manifestly impossible to make an accurate determination of the free period by determination of the damped period and applying the correction for damping. The difference between the amount measured and the limit of accuracy of measurement is too slight. If then we wish to be able to determine the accuracy with which the galvanometer will record periodic motions of any frequency or to be in position to apply corrections to registered curves, we must measure τ_0 by the method indicated above. Having determined the value of τ_0,

[4] But one experiment was performed. The very close correspondence of the results is probably in part accidental, but making due allowance for this, the result indicates the possibility of satisfactory accuracy in determining τ_0 from a critically damped deflection.

and the strength of the effective magnetic field we are in possession of numerical values of all of the quantities in equation (6). The possible advantage of having these values will become apparent presently.

We shall next discuss condition (c), damping more than critical. The auxiliary equation now has two real roots,

$$\beta_1 = -\frac{k}{2m} + \sqrt{\frac{k^2}{4m^2} - \frac{s^2\pi^2a^2}{l^2}}$$

$$\beta_2 = -\frac{k}{2m} - \sqrt{\frac{k^2}{4m^2} - \frac{s^2\pi^2a^2}{l^2}}$$

Writing β_1, β_2 instead of the longer equivalent expressions, the solution of (7) for condition (c) is,

$$u = \frac{2}{l}\sum_{s=1}^{s=\infty} \sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}\,d\lambda \left| e^{\beta_2 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right) - e^{\beta_1 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right)\right| \quad (16)$$

(See appendix, h).

This solution satisfies all of the boundary conditions. It is zero when x is zero and when x equals l and it is equal to $F(x)$ when $t=0$. Its first derivative with respect to t vanishes when t is zero and on differentiating it and substituting in the differential equation, it is found to satisfy it. To (16) must be added the same particular integral which occurs in solution (8) for the condition (a) if it is desired to express the response to a periodic impressed force. No convenient analytical expression can be given for determination of the ratio of the actual to the theoretical deflection for a given impressed force when the galvanometer is overdamped. It has been shown how all of the quantities in the expressions for β_1 and β_2 may be determined. If their numerical values be substituted in the amplitude term of the particular integral the ratio of this to the amplitude term for the same tension, but without mass and damping can be readily calculated.

If the string of the galvanometer be sufficiently slackened, a tension will at last be reached so small that the increase in tension due to deflection can no longer be neglected. When this condition is reached it will be recognized by the fact that the deflections, hitherto proportional to the strength of current over a considerable range now lose their proportionality except for a very small range. It will also be noticed that as this condition is approached a small

SEE APPENDIX A, PART 2

change in the position of the micrometer screw produces a relatively large change in the sensitivity of the instrument. If the tension be still further decreased, the deflections again become porportional to the strength of current, the galvanometer becomes very sensitive to small currents and its deflections very slow compared with their period under greater tension. The restoring force is now due to the action of gravity, modified slightly by the stiffness of the string which can no longer be neglected. The constancy of zero and the strictness of proportionality of deflections to the strength of current in this condition are remarkable and were pointed out by Einthoven (3) p. 317. The preceding analysis does *not* apply in any manner whatever to this condition. It will be evident that, neglecting the effect of stiffness of the string, its form when undeflected will be a catenary and the tension will vary according to the well known law of the catenary, being greatest at the upper end and least at the bottom. This suggests a novel construction for a string galvanometer intended to be used only with the string in this slackened condition for measurements of the smallest currents capable of producing measurable deflections. The microscopes should be placed *under* the pole-pieces and if it were not for the stiffness of the string they should be as near the lower end as possible, but as the stiffness is certain to affect the motion in this condition of the instrument, the position of choice will be somewhat above the lower end. An analysis making allowance for the stiffness of the string and other peculiar features of this condition would appear to offer considerable difficulties and it may be easier to investigate the matter experimentally. I hope to have an opportunity to do this soon, perhaps before this series of papers is completed. Einthoven was able to attain a sensitivity of 10^{-11} ampere per mm with his galvanometer and the writer has duplicated this with a copy of Professor Einthoven's instrument in the Physiological Laboratory at Columbia University. If the modification above suggested should make possible an increase of this sensitivity by one or more powers of ten as seems not beyond possibility, the instrument might become available for work which has hitherto required a sensitive moving magnet type or an electrometer method.

3. THE PARTIAL VIBRATIONS OF THE STRING

We now proceed to investigate the effect on the partial modes of the string's motion of the particular form assumed by the string

when deflected by steady continuous current, this form depending upon the distribution of the fixed magnetic field. We shall consider first the circular arc which would be the form if the field were actually uniform throughout. The equations for this distribution of the field have already been derived by Crehore (loc. cit.), but we shall derive them more readily by substituting the equation of the circular arc in the expression for $f(\lambda)$ in the solution of the partial differential equation. The equation of the circular arc itself presents some analytical difficulties and we shall follow Crehore's procedure in substituting the equation of a simpler conic which closely approximates the shape of the circular arc. We shall adopt a slightly different procedure in this development from that employed by Crehore as being perhaps more easily followed by the casual reader. In Fig. 4, OL is the string of length, l, and the origin of coordinates

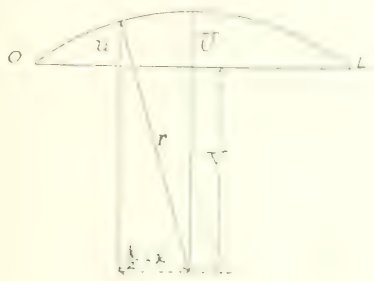

FIG. 4.

is taken at the end 0. U is the steady deflection of the middle point when the string is in equilibrium with steady current flowing. u is the ordinate of any point, x. The equation of the circular arc is

$$\left(\frac{l}{2}-x\right)^2+(u+V)^2=r^2=(U+V)^2$$

or,

$$\frac{l^2}{4}-lx+x^2+2uV+u^2+V^2=U^2+2UV+V^2$$

U is a small quantity and u is either equal to U or a still smaller quantity. (See appendix c). Also r and V differ from each other by the small quantity U, in comparison with which they are both large. For the purpose of deriving the equation of a curve which shall sensibly coincide with the circular arc and still be of such form as to be easily handled, we may consider r and V as equal to each other and drop u^2 and U^2 as small quantities of the second order. We

recall that we have derived equation (3), $r=\frac{l^2}{8U}$, and this we now write equal to V. Then,

$$\frac{l^2}{4}-lx+x^2+2uV=\frac{2Ul^2}{8U}=\frac{l^2}{4}$$

$$x^2-lx=-2uV\ ,$$

or

$$u=-\frac{1}{2V}(lx-x^2) \tag{17}$$

or since $V=\frac{l^2}{8U}$,

$$u=\frac{4U}{l^2}(lx-x^2) \tag{18}$$

Equation (8) may be looked upon as a symbolic solution of the equation of motion. It applies to any form whatever which the deflected string may take. Considering only that part of (8) which has to do with the shape of the string, namely that which we have previously referred to as $F(x)$, we may substitute for $f(\lambda)$ the function defined by (18), writing as already explained λ instead of x to indicate that it is a function of the limits of the integration. We shall then have an expression which we may integrate to get the Fourier series which defines the shape of the string (to the same order of approximation that (18) corresponds to the circular arc) when the field is uniform. We have then,

$$F(x)=\frac{2}{l}\sum_{s=1}^{s=\infty}\sin\frac{s\pi x}{l}\int_0^l\frac{4U}{l^2}(l\lambda-\lambda^2)\sin\frac{s\pi\lambda}{l}d\lambda\ ,$$

or

$$F(x)=\sum_{s=1}^{s=\infty}\sin\frac{s\pi x}{l}\cdot\frac{8U}{l^3}\left[l\int_0^l\lambda\sin\frac{s\pi\lambda}{l}d\lambda-\int_0^l\lambda^2\sin\frac{s\pi\lambda}{l}d\lambda\right] \tag{19}$$

Performing the integrations indicated in (19), (See appendix, i), we have,

$$F(x)=\sum_{s=1}^{s=\infty}\frac{32U}{s^3\pi^3}\sin\frac{s\pi x}{l} \tag{20}$$

or,

$$F(x)=1.033U\left(\frac{\sin\pi x}{l}+\frac{1}{3^3}\sin\frac{3\pi x}{l}+\frac{1}{5^3}\sin\frac{5\pi x}{l}+\cdots\cdots\right) \tag{21}$$

a rapidly convergent series. The movement of the string can be

observed only at the middle point, that is at the point $x = \frac{l}{2}$, and $\sin\frac{s\pi\frac{l}{2}}{l}$ equals $(1-1+1-1+1-\cdots)$ for all odd values of s and zero for all even values.

For the middle point of the string we have,

$$F\left(\frac{l}{2}\right) = 1.033U - .038^2U + .008^2U - .003U + \cdots \quad (22)$$

This series converges so rapidly that unless the numerical work is carried to a greater number of decimal places, the series is sensibly equal to U after four terms. It is seen that the third partial, the first which can occur, is nearly four per cent of U.

If the field distribution were such that the string when deflected by steady continuous current assumed the form,

$$u = \sin\frac{\pi x}{l}$$

we should have, $F_2(x) = U \sin\frac{\pi x}{l}$ and $F_2\left(\frac{l}{2}\right) = U$. Under this condition the motion of the string would be the simplest possible. No partial vibrations would occur; all points of the string would move in the same phase and the string would remain a simple curve of sines until all motion ceased. Crehore, *loc. cit.* p. 223, discusses this matter and suggests that the ideal construction for a string galvanometer would be that in which the pole faces are so shaped that the field is strongest in the middle and diminishes in strength toward either end according to a sine law. This he shows would result in deflection into a simple curve of sines. This would indeed be ideal, but from the constructional standpoint it appears to be impracticable. Only the greatest care in manufacture will insure that approach to accurate parallelism of the pole faces upon which the lateral uniformity of the field depends and upon which in turn depends the proportionality of the deflections to the strength of current. If in addition an attempt were to be made to alter the face lengthwise, still maintaining parallelism in the other direction, the manufacturing would become a matter of considerable complexity. This might be overcome if the results were to be enough better to justify it, but a further difficulty arises from the fact that the shape would have to be altered for different specimens of iron and finally no way has been suggested whereby the maker could control

the process so as to determine when the ideal condition has been realized.

Since any other field distribution whatever will introduce partial modes into the movement of the string, it becomes a matter of moment to ascertain as nearly as we may their probable magnitude under actual conditions and their probable effect on measurements made with the galvanometer. The only theoretical discussion of the string galvanometer with which the writer is familiar which has taken account of the partials at all is that of Crehore and he has considered them only for the assumption of uniformity of the field. Since the amplitudes of the partials are small compared with that of the fundamental it is evident that any conclusion drawn from an assumption which we know to be at variance with the facts is apt to prove illusory. Before we attempt to consider the possible effect of such partials as equation (22) shows would be present if the field were uniform we must first show that the difference between (22) and a series which represents more accurately the form for the actual distribution of the field is not greater than the difference between (22) and $F_2(x) = U \sin \frac{\pi x}{l}$.

By exercise of care in construction the lateral distribution of the field can be kept of such uniformity that the deflections of the string are proportional to the strength of current over a range of movement greater than that usually employed for measurements. Since the same general order of mechanical accuracy is attained in the dimension at right angles to the direction of motion of the string, we may reasonably infer a corresponding uniformity of field in this direction except for the region of the perforation made to admit the optical system. The effect of the projecting ends of the string we may neglect since the amount of projection can be kept small and the motion near the ends is very small in any event. In order to obtain a limiting value for the possible effect of the weakening of the field at the perforation we may make the somewhat violent assumption that the field in this region is zero. For a given type of optical system the effect of the perforation will be greater the shorter the string and we shall therefore consider the smaller of the two instruments to be later described, as typical in order to get the worst result likely to be encountered. In this instrument the string length is about six times the diameter of the perforation and for greater simplicity of the analysis we shall take it as exactly so.

We shall assume that the field is uniform from either end of the string as far as the perforation. The accompanying figure 5 will make clear the condition assumed.

The end portions of the string in the uniform parts of the field will be deflected into circular arcs and the central portion of length $\frac{l}{6}$ will be dragged out by the motion of the end portions, but will remain straight. To express the shape of the string on this assumption in an equivalent Fourier series we may proceed in either of two ways. We may obtain series for the portions OAB and DLC and add to these a series for the rectangle $ABCD$. This which is the more direct method is somewhat laborious. It is actually simpler to find a series for the figure made up of the rectangle and the arc BMC, then an expression for the rectangle. Subtracting the series for the rectangle from that for the figure $ABMCD$, we have left a series

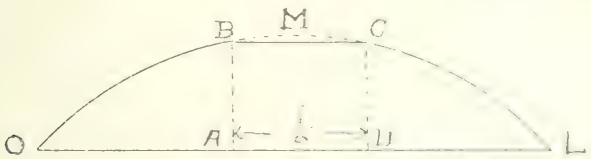

FIG. 5

for the small segment included between the arc BMC and the rectangle. On subtracting this last series from that which defines the shape of the string when the field is uniform throughout we have left a series representing the string in a field uniform except for the middle sixth, which is zero. For simplicity we shall in every case employ a few terms of the series when $x = \frac{l}{2}$ since it is the value of the amplitudes of the various partials at the middle point of the string which affects the record. Before we can carry out this analysis we require to determine what fraction the ordinate AB is of I, the ordinate of the middle point of the arc when the field is uniform throughout. The flat portion BC owes its position entirely to forces acting on the portions of the string OB and CL. If the field were uniform throughout we could find the part of its deflection which is due to the reaction between the middle sixth of the fixed field and the field around the middle sixth of the string due to the current I by assuming this middle portion to be clamped fast at the points $\frac{5l}{12}$ and $\frac{7l}{12}$, the tension on it remaining the same as when

these points are free. The radius of curvature of this central portion will be identical with that of the string considered as a whole. We consider its ends fixed at the points B and C and require to find the deflection U_2 of its middle point above the line BC in terms of the deflection U of the entire string in uniform field above the line OL. By equation (3) we have $r=\frac{l^2}{8U}$. Then for the portion BMC, $r=\frac{\left(\frac{l}{6}\right)^2}{8U_2}$. Equating these two expressions for r, we have

$$\frac{l^2}{U}=\frac{\left(\frac{l}{6}\right)^2}{U_2} \text{ or } l^2U_2=\frac{l^2}{36}U$$

whence,

$$U_2=\frac{U}{36} \tag{23}$$

If we write C for the height of the ordinate AB, $C=\frac{35}{36}U=.97\ U$.

In an uniform field, the Fourier series for the part of the circular arc lying between $x=\frac{5l}{12}$ and $x=\frac{7l}{12}$ is given by

$$F_m(x)=\sum_{s=1}^{s=\infty}\frac{8U}{l^3}\int_{\frac{5l}{12}}^{\frac{7l}{12}}(l\lambda-\lambda^2)\sin\frac{s\pi\lambda}{l}d\lambda\sin\frac{s\pi x}{l}. \tag{24}$$

On performance of the indicated integrations, (Appendix j), this gives,

$$F_m(x)=\sum_{s=1}^{s=\infty}\left\{\frac{32U}{s^3\pi^3}\sin\frac{s\pi}{12}-\frac{8U}{3s^2\pi^2}\cos\frac{s\pi}{12}+\frac{280U}{72s\pi}\sin\frac{s\pi}{12}\right\}\sin\frac{s\pi x}{l} \tag{25}$$

On performance of indicated numerical operations, (25) further reduces to

$$F_m(x)=\sum_{s=1}^{s=\infty}\left\{\frac{1.033\,U}{s^3}\sin\frac{s\pi}{12}-\frac{.2704\,U}{s^2}\cos\frac{s\pi}{12}+\frac{1.24\,U}{s}\sin\frac{s\pi}{12}\right\}\sin\frac{s\pi x}{l} \tag{26}$$

We must now obtain a series for the rectangular constant portion, $F_C(x)=C$. This is given by,

$$F_C(x)=\frac{2}{l}\sum_{s=1}^{s=\infty}\int_{\frac{5l}{12}}^{\frac{7l}{12}}C\sin\frac{s\pi\lambda}{l}d\lambda\sin\frac{s\pi x}{l} \tag{27}$$

which may be written,

$$F_C(x) = \frac{2}{l} C \sum_{s=1}^{s=\infty} \int_{\frac{5l}{12}}^{\frac{7l}{12}} \sin\frac{s\pi\lambda}{l} d\lambda \sin\frac{s\pi x}{l} = \sum_{s=1}^{s=\infty} \frac{4C}{s\pi} \sin\frac{s\pi}{12} \sin\frac{s\pi x}{l} \quad (28)$$

If we put for C, its value, .97 U, and for x, $\frac{l}{2}$, we have to four terms,

$$F_C\left(\frac{l}{2}\right) = .3198U - .2910U + .2386U - .1704U + \cdots \quad (29)$$

This series converges toward the value $C = .97\ U$, and it is evident that the convergence is slow. Taken in connection with the series for the circular arc, it will, despite its slow convergence, give us an idea of the magnitude of the lower partials and show whether they tend to remain of large value. From (26), putting $x = \frac{l}{2}$, we obtain,

$$F_m\left(\frac{l}{2}\right) = .3275U - .2980U + .2447U - .1754U + \cdots \quad (30)$$

Subtracting (29) from (30),

$$F_m\left(\frac{l}{2}\right) = .3275U - .2980U + .2447U - .1754U + \cdots$$

$$F_C\left(\frac{l}{2}\right) = .3198U - .2910U + .2386U - .1704U + \cdots$$

$$\psi\left(\frac{l}{2}\right) = .0077U - .0070U + .0061U - .0050U + \cdots \quad (31)$$

$\psi\ (x)$ represents the small circular arc BMC in Fig. 5 and, subtracting (31) from (22),

$$F\left(\frac{l}{2}\right) = 1.033U - .0382U + .0082U - .003U + \cdots$$

$$\psi\left(\frac{l}{2}\right) = .0077U - .0070U + .0061U - .0050U + \cdots$$

$$F\left(\frac{l}{2}\right) - \psi\left(\frac{l}{2}\right) = 1.0253U - .0312U + .0021U + .0020U + \cdots \quad (32)$$

Equation (32) gives the amplitudes at the middle point of the string, of the fundamental and of the third, fifth, and seventh partials, supposing the field uniform except for its middle sixth,

which is of strength zero. It will be observed that the third partial is a little smaller than the third of (22), the series for uniform field.

Although the convergence of (31) is not rapid, we may be certain that no term after the fourth will be larger than the fourth and as the series (22) converges very rapidly, there will be no single partial larger than those already computed. It appears then, perhaps unexpectedly, that weakening of the central part of the field results in less disturbance from partials than would be the case if the field were perfectly uniform.

In the actual instrument the field in the region of the perforation is by no means zero. Measurements made by Einthoven on his original instrument showed the field in this region to be somewhat less than half the strength measured in the stronger end regions. The diameter of the perforation was much larger and the length of the air gap much greater in his instrument than in the one on which the present computations are based, so that we shall perhaps not be far from the truth if we assume the field strength in the region of the perforation to be half that in the end regions. Naturally it is not uniform throughout the region of the perforation, but the effect of this non-uniformity will be of the second order. We may then arrive at a series which represents with some approach to accuracy the real magnitude of the partials in this instrument by subtracting from (22) one-half the value of the series (31) (we might, of course get the same result by *adding* half of (31) to (32).)

$$F\left(\frac{l}{2}\right) = 1.033U - .0382U + .0082U - .003U + \cdots \qquad (22)$$

$$\tfrac{1}{2}\psi\left(\frac{l}{2}\right) = \quad .00385U - .0035U + .00305U - .0025U + \cdots \qquad (33)$$

$$F\left(\frac{l}{2}\right) - \tfrac{1}{2}\psi\left(\frac{l}{2}\right) = 1.02915U - .0347U + .00515U - .0005U \qquad (34)$$

The third partial in (34) approaches that in (22) as might have been expected, but it is smaller. We may then safely assume that in this galvanometer the largest partial will be between three and four per cent of the maximum steady current deflection.

For precise measurements the string galvanometer should be used in the critically damped condition and a discussion of the partials is pertinent only where precise measurements are in question so that it may be instructive to develop the equation which applies to the motion of the partials when the fundamental is critically

damped. For critical damping of the fundamental we remember that the condition is $\frac{\pi a}{l}=\frac{k}{2m}$ and for critical damping of the sth partial the condition is $\frac{s\pi a}{l}=\frac{k}{2m}$. Here the damping factor must be larger than would suffice to critically damp the fundamental. The occurrence of s as a factor in the restoring force term indicates that for any given amplitude the amount of energy stored in the deflected string is greater according as the segment under consideration is shorter, which is in entire agreement with the well known experimental fact that under a given tension a shorter string of the same mass etc. requires the application of greater force to produce a given deflection as compared with a longer one. When therefore the fundamental is critically damped, the partials will all be underdamped and to them the oscillatory form of the solution, equation (8), still applies. To investigate the motion of the partials when the fundamental is critically damped, we may put $\frac{\tau_0}{t}$ or its equivalent $\frac{2\pi}{\tau_0}$ for $\frac{k}{2m}$ wherever the latter occurs in (8). On making this substitution and reducing, (See appendix, I.), we have for the complementary function,

$$u=\frac{2}{l}e^{-\frac{2\pi t}{\tau_0}}\sum_{s=1}^{s=\infty} s\sqrt{\frac{1}{s^2-1}}\cos\left(\frac{2\pi t}{\tau_0}\sqrt{s^2-1}\right.$$

$$\left.-\tan^{-1}\frac{1}{\sqrt{s^2-1}}\right)\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda$$

$e^{-\frac{2\pi t}{\tau_0}}$ is a factor of the entire expression and defines the law of decay of the motion of the partials. Each will die away in a series of oscillations whose frequency will depend on the order of the partials. They will all die away at the same rate and this will be the same as the rate of decay of the fundamental. The only difference is that the fundamental will die away without oscillations and partials of higher order will perform a greater number of oscillations before their motion stops than will those of lower order. At first sight it might seem that a partial of amplitude from three to four per cent of the total deflection of the string would make itself very evident in the record. Let us consider the amplitude of the third partial at the time $t=\frac{\tau_0}{4}$. Its amplitude will have declined to, $n=e^{-\frac{\pi}{2}}=.208$

SEE APPENDIX A, PART 2

of its initial amplitude. It is usual practice to employ photographic plates or film not over 6 cm wide, admitting of a double deflection of 6 cm. Let us suppose that the initial deflection was 3 cm. We are here speaking of the deflection of the magnified shadow, magnification assumed to be 1000 diameters. The initial amplitude of the third partial we may take to be four per cent of the total deflection, which would make it 1.2 mm. Then in a time from the beginning of the motion equal to one quarter the natural period of the string, it will have decayed to .25 mm, or just above the limit of legibility. Its own undamped period is $\frac{\tau_0}{3}$ so that it will have performed less than one complete oscillation before it has decayed to this small value. It has already been shown in connection with the discussion of the curve, Fig. 3, that in order to secure accuracy of the amplitude to one per cent, it is necessary that the free period of the critically damped galvanometer be one tenth the period of the highest component of the impressed force which it is desired to measure with this accuracy. If the period of the fundamental were .001 second, the instrument would be adapted to record with one per cent accuracy up to a period of .01 second. In these circumstances a speed of one meter per second for the photographic plate or film would be ample to resolve the motion and at this speed the record will have traveled but one quarter of a millimeter during the time that the third partial is decaying to an amplitude of a quarter of a millimeter. The amplitude will have diminished to nearly the limit of legibility about the time that the travel of the plate or film is sufficient to permit of reading. The major part of the motion of the partial will have died away during a time so short that it is not resolved by the motion of the plate. In actual practice it is likely that a record involving frequencies not to exceed 100 per second would be made at less than one meter per second, in which case the chance of seeing anything of the third partial would be still smaller. I have never seen anything in the record of motion of a critically damped quartz fiber which I could identify as probably due to partials. They become easily evident, however, in the motion of a duraluminum wire .04 mm in diameter, even when the damping is somewhat more than critical. The third partial could be identified with certainty when the fundamental period was .003 second. Why the partial should be evident in the record of motion of this wire and not in records made with coated quartz fibers does not appear

from the equations. One notable difference between wire and fiber is in the flexibility of the latter. The equations do not take account of rigidity.[5] If rigidity has the effect of increasing the magnitude of the partials this should be borne in mind when somewhat stiff metallic wires are employed for strings as is occasionally done.

If a rather heavy quartz string is put under sufficient tension it can be made to execute several damped oscillations before it comes to rest. If the amplitudes of successive oscillations are compared it is found that the decrement is uniform after the first oscillation, but a value based on comparison between the first and second does not agree with the others. This was noticed, but not explained by Einthoven, (Weitere Mitteilungen über das Saitengalvanometer, Annalen der Physik, 4th Series, 21, p. 490; 1906), and Crehore comments on it (loc. cit. p. 222) giving the explanation. The discrepancy is due to the partials which have not died away during the first oscillation of the fundamental, but have become imperceptible by the time the second and third fundamental oscillations occur. The galvanometer is not used in this condition and the only purpose for which these oscillations would be measured would be to determine the logarithmic decrement; either for determination of the damping constant, or the correction for damping to be applied in obtaining the free period from the damped period. We have shown how the free period and damping constant can be determined in a manner capable of giving more accurate results and dismiss the matter with the remark that it is of interest as indicating the existence of the partials.

There is one aspect of the partials which we should not pass over without investigation. That is the possibility that a component of the impressed force may happen to agree in period with one of the partials so that resonance of that partial may occur. The significance of such an occurrence will depend on the sharpness of the resonance, which in turn depends upon the amount of damping. We may examine this matter by studying the particular integral which defines the forced motion under the assumptions, first that the damping of the fundamental is critical, since this is the condition of principal interest, and second, that n_{sk} in the particular integral is equal to $2\pi f_{sk}$, where f_{sk} is the damped frequency of the sth. partial. Since the sth. partial is subject to considerable damp

[5] For a hint as to the method to be followed if it is desired to consider rigidity in the analysis, see Lord Rayleigh, Theory of Sound, *I*, Art. 137, p. 163; 1877.

ing when the fundamental is critically damped, we cannot derive an entirely accurate result by substituting for $f_{s,k}$, $\frac{s}{\tau_0}$, the undamped frequency of the sth. partial, but must derive an expression for the damped period of the partial under the given condition of damping. The damped period is given by,

$$\tau_{s,k} = \frac{2\pi}{\sqrt{\frac{s^2\pi^2a^2}{l^2} - \frac{k^2}{4m^2}}}$$

and for critical damping of the fundamental we have as usual,

$$\frac{\pi a}{l} = \frac{k}{2m} = \frac{2\pi}{\tau_0},$$

so that,

$$\tau_{s,k} = \frac{2\pi}{\sqrt{\frac{4\pi^2s^2}{\tau_0^2} - \frac{4\pi^2}{\tau_0^2}}} = \frac{\tau_0}{\sqrt{s^2-1}}$$

and $f_{s,k} = \frac{\sqrt{s^2-1}}{\tau_0}$ so that $n\omega = \frac{2\pi\sqrt{s^2-1}}{\tau_0}$ and $n^2\omega^2 = \frac{4\pi^2s^2-4\pi^2}{\tau_0^2}$.

We have also from the damping condition that $k = \frac{4m\pi}{\tau_0}$ and $k^2 = \frac{16m^2\pi^2}{\tau_0^2}$ and substituting these values in the amplitude factor of the particular integral of (8), we have,

$$\frac{HI_s}{\sqrt{\frac{16m^2\pi^2}{\tau_0^2} \times \frac{4\pi^2s^2-4\pi^2}{\tau_0^2} + m^2\left(\frac{4\pi^2s^2}{\tau_0^2} - \frac{(4\pi^2s^2-4\pi^2)}{\tau_0^2}\right)^2}}$$

This by simple algebraic transformations readily reduces to,

$$\frac{HI_s\tau_0^2}{4\pi^2m\sqrt{4s^2-3}} \tag{36}$$

Perhaps the most convenient way to appreciate the meaning of (36) will be to determine the ratio between the amplitude which it expresses and that which an equal force of the same period would produce if it acted on an ideal instrument with massless string of length, l, and without damping. The amplitude factor of such an instrument may be derived from (12) by putting $s = 1$, and is, $\frac{HI_sl^2}{\pi^2T}$. The deflections of this ideal instrument will be the same for all frequencies as the deflection of an actual instrument under the same conditions of field strength and tension if the maximum amplitude

of the periodic current were to flow as a steady current through it for an indefinitely long time. Dividing (36) by the expression derived from (12), we have,

$$\frac{U_{sk}}{\underline{U}} = \frac{HI_s\tau_0^2}{4\pi^2 m\sqrt{4s^2-3}} \times \frac{\pi^2 T}{HI_s l^2} = \frac{T\tau_0^2}{4ml^2\sqrt{4s^2-3}}$$

Remembering that $\frac{T}{m} = a^2$, and that $\frac{\tau_0^2}{4} = \frac{l^2}{a^2}$, we have,

$$\frac{a^2 l^2}{l^2 a^2\sqrt{4s^2-3}} = \frac{1}{\sqrt{4s^2-3}} = \frac{U_{sk}}{\underline{U}} \qquad (37)$$

The following table gives the value of this ratio for several orders of s and, for comparison, values of the deflection of the critically damped fundamental for the same period of impressed force, taken from the curve, Fig. 3.

Order of partial	Ratio $\frac{U_{sk}}{\underline{U}}$	Ratio $\frac{U}{\underline{U}}$
3	.174	.10
5	.1016	.04
7	.072	.02
9	.0558	.012

From this table it appears that the resonance of the partials when the damping is of sufficient amount to make the motion of the fundamental just non-oscillatory, is not at all sharp. The deflections are much less than the fundamental steady current deflection for a current equal to the maximum amplitude of the periodic current. It is, however, always distinctly greater than the fundamental amplitude of the same instrument for the same frequency and this discrepancy increases with the order of the partial as we might have expected. Distortion due to this cause will then be slight, and values corrected according to the curve, Fig. 3, will in general meet practical requirements. If harmonic analysis of a record shows the occurrence of a frequency in resonance with a partial and it is desired to apply a more rigorous correction, the equations just developed indicate the method to be followed.

This completes the purely theoretical part of this paper. In Part II we shall consider the constants of actual instruments and discuss the limits of period, damping and sensitivity which can be practically attained with different types. We shall have occasion to refer to calculations on the magnetic circuits of string galvanome-

ters, but it is unnecessary to discuss this matter in its theoretical aspects since there is nothing relating to the magnetic circuit of the string galvanometer of a special character.

APPENDIX TO PART I

(a)

For a more complete mathematical discussion of this part of the subject, see Thomson and Tait, Treatise on Natural Philosophy, Impression of 1912, Part II, Articles 574-577, inclusive, pp. 118-122.

(b)

The curve into which the string is deflected by steady current has been assumed by different writers to be a parabola, catenary, etc. Crehore was the first to show clearly that it is a circle if the field is uniform. This might also have been inferred from experimental results. For example Wiedemann [9], describes an experiment in which a platinum wire placed in an uniform field and carrying current of sufficient strength to make it soft, deflects into a semicircle. Wehnelt [10], showed that a stream of soft cathode rays in an uniform magnetic field is bent into a complete circle whose radius depends on the field strength and on the velocity of the electrons.

(c)

Referring to Fig. 2, we see from the properties of a right triangle that, $r^2-\left(\frac{l}{2}\right)^2=(r-U)^2$ or $r^2-\frac{l^2}{4}=r^2-2rU+U^2$. If the length of the string be taken as 7 cm and the deflection, U, as .003 cm, which may be regarded as representative and not as extreme values, r is of the order of 20 meters and l^2 is a little more than one part in two hundred million of r. It is about five parts in a million of l^2 so that in comparison with either r or l^2 we may neglect U^2. Dropping U^2, $2rU=\frac{l^2}{4}$, and $r=\frac{l^2}{8U}$, which is (3).

(d)

Equation (7) is so well known that it seems hardly worth while to go into its derivation. The reader may consult, Rayleigh, Theory of Sound, vol. I, p. 135 and 149, edition of 1877; or Houstoun, Introduction to Mathematical Physics, pp. 109 to 113, inclusive.

The complementary function of the solution, (8), is taken from Byerly's Fourier's Series and Spherical, Cylindrical and Ellipsoidal Harmonics, Page 115, equation (14). The notation used in this paper is slightly different and the equation has been transformed

to an equivalent form better adapted to the purposes of this paper. The solution as given by Professor Byerly, but in the notation used throughout this paper is,

$$u=\frac{2}{l}e^{-2m}\sum_{s=1}^{s=\infty}\left[\left(\cos t\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}+\frac{k}{2m\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}}\sin t\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}\right)\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}d\lambda\right] \quad (8a)$$

The terms in the parenthesis are of the form, $A\sin\theta+B\cos\theta$.

Consider the right triangle, Fig. 6, and let ϵ be the angle opposite the side A. $\sin\epsilon=\frac{A}{\sqrt{A^2+B^2}}$, $\cos\epsilon=\frac{B}{\sqrt{A^2+B^2}}$ and $\tan\epsilon=\frac{A}{B}$. Put $Q=\sqrt{A^2+B^2}$. Then $Q\sin\epsilon=A$ and $Q\cos\epsilon=B$. Substitute in the expression $A\sin\theta+B\cos\theta$, $Q\sin\epsilon$ for A, and $Q\cos\epsilon$ for B. The expression becomes, $Q(\sin\epsilon\sin\theta+\cos\epsilon\cos\theta)$. This by a well known trigonometrical substitution becomes, $Q\cos(\theta-\epsilon)$. $\epsilon=\tan^{-1}\frac{A}{B}$.

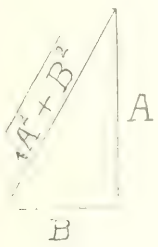

FIG. 6

(See S. L. Loney's Plane Trigonometry, Table of Formulae, p. xi, Group VI, No. 4, or B. O. Peirce, Short Table of Integrals, p. 75, No. 592.) The same transformation is used elsewhere in this paper.

In equation (8a) we may write for A, $\frac{k}{2m\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}}$ and for B, unity.

Q will then be, $\sqrt{1+\frac{k^2}{\frac{4m^2s^2\pi^2a^2}{l^2}-k^2}}$ which by a simple algebraic transformation gives, $Q=\sqrt{\frac{1}{1-\frac{l^2k^2}{4m^2s^2\pi^2a^2}}}$, and

$\epsilon = \tan^{-1} \dfrac{k}{2m\sqrt{\dfrac{s^2\pi^2a^2}{l^2} - \dfrac{k^2}{4m^2}}}$. We may now write for (8a),

$$u = \frac{2}{l} e^{-\frac{kt}{2m}} \sum_{s=1}^{s=\infty} \sqrt{\frac{1}{1 - \dfrac{l^2k^2}{4m^2s^2\pi^2a^2}}} \cos\left(t\sqrt{\frac{s^2\pi^2a^2}{l^2} - \frac{k^2}{4m^2}} - \tan^{-1} \frac{k}{2m\sqrt{\dfrac{s^2\pi^2a^2}{l^2} - \dfrac{k^2}{4m^2}}} \right) \sin\frac{s\pi x}{l} \int_0^l f(\lambda) \sin\frac{s\pi\lambda}{l} d\lambda$$

which is the complementary function of (8).[6]

As regards the method of obtaining the particular integral of (8), we may remark that there is a close formal analogy between the methods applicable for obtaining a particular integral of an ordinary differential equation and those which apply to partial differential equations. The reader may consult, A Treatise on Ordinary and Partial Differential Equations, by W. W. Johnson, p. 109, Art. 111 et seq. for methods applicable to the ordinary form containing trigonometric functions and for the corresponding methods for

[6] To attempt to ascertain the form of the *most general solution* of a partial differential equation by generalization from the corresponding ordinary differential equation is admittedly futile. (See A. R. Forsyth, Theory of Differential Equations, Part IV, Vol. V, pp. 2-4.) In the applications of these equations to mathematical physics, however, the most complete solution is apt to be much too general and must often be limited by boundary conditions dependent on physical specifications quite unrelated to the form of the equation. In solving the equation (7) for the conditions of critical and more than critical damping, we shall find that such a generalization will lead to solutions of sufficient generality for our purpose. We might have derived the solution for the oscillatory case in this manner also. This alternative method of solution is given below.

The solution of, $\ddot{u} + \frac{k}{m}\dot{u} + \frac{\mu}{m}u = 0$ when $\frac{\mu}{m} > \frac{k^2}{4m^2}$, is,

$$u = e^{-\frac{kt}{2m}}\left(A\sin t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} + B\cos t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} \right) \qquad (1)$$

To evaluate the arbitrary constants, A and B, we have, that when $t=0$, $u=U$ and $\dot{u}=0$. Putting $t=0$, $u=e^{0}(B)=U$. Differentiating (1),

$$\dot{u} = -\frac{k}{2m}e^{-\frac{kt}{2m}}\left(A\sin t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} + B\cos t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} \right) + e^{-\frac{kt}{2m}}\left(A\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}\cos t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} - B\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}\sin t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} \right) = 0$$

when $t=0$

partial differential equations, p. 357, Art. 330. For another method, see A. R. Forsyth, Treatise on Differential Equations, p. 74, 111, for the ordinary form and p. 518, Art. 264, for partial equations.

Putting t equal to zero in this expression, we have,

$$\dot{u} = -\frac{k}{2m}B + A\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}, \text{ or, since } B = U,$$

$$A = \frac{kU}{2m\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}}$$

The solution then is,

$$u = U e^{-\frac{kt}{2m}}\left(\frac{k}{2m\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}}\sin t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}} + \cos t\sqrt{\frac{\mu}{m} - \frac{k^2}{4m^2}}\right)$$

To solve the corresponding partial differential equation we must substitute for U, a function of x which will satisfy the same conditions as U and at the same time the further conditions that the deflection is always zero when $x=0$ and when $x=l$ and that when $t=0$ the shape of the string is represented by some Fourier series. Such a function is,

$$U(x) = \frac{2}{l}\sum_{s=1}^{s=\infty}\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}\,d\lambda.$$

Substituting this for U, we have as a solution of,

$$\frac{\partial^2 u}{\partial t^2} = \frac{k}{m}\frac{\partial u}{\partial t} - c^2\frac{\partial^2 u}{\partial x^2} = 0,$$

$$u = \frac{2}{l}e^{-\frac{kt}{2m}}\sum_{s=1}^{s=\infty}\left[\left(\frac{k}{2m\sqrt{\frac{s^2\pi^2c^2}{l^2} - \frac{k^2}{4m^2}}}\sin t\sqrt{\frac{s^2\pi^2c^2}{l^2} - \frac{k^2}{4m^2}}\right.\right.$$

$$\left.\left. + \cos t\sqrt{\frac{s^2\pi^2c^2}{l^2} - \frac{k^2}{4m^2}}\right)\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}\,d\lambda\right]$$

which is the equivalent of Professor Byerly's solution and readily reduces to,

$$u = \frac{2}{l}e^{-\frac{kt}{2m}}\sum_{s=1}^{s=\infty}\left[\sqrt{\frac{1}{1 - \frac{k^2}{4m^2s^2\pi^2c^2/l^2}}}\cos\left(t\sqrt{\frac{s^2\pi^2c^2}{l^2} - \frac{k^2}{4m^2}}\right.\right.$$

$$\left.\left. - \tan^{-1}\frac{k}{2m\sqrt{\frac{s^2\pi^2c^2}{l^2} - \frac{k^2}{4m^2}}}\right)\sin\frac{s\pi x}{l}\int_0^l f(\lambda)\sin\frac{s\pi\lambda}{l}\,d\lambda\right]$$

The reason for writing $\frac{s^2\pi^2a^2}{l^2}$ in place of $\frac{\mu}{m}$ of the ordinary equation will be apparent to anyone who is familiar with the usual method of solving this equation.

Writing D for $\frac{\partial}{\partial t}$, and D' for $\frac{\partial}{\partial x}$, we may express the particular integral of (7) symbolically, thus:

$$u = -\frac{HI_n}{m\left(D^2+\frac{k}{m}D-a^2D'^2\right)}\sum_{n=1}^{n=\infty}\sum_{-1}^{-\infty}\cos n\omega t\sin\frac{s\pi x}{l}.$$

Omitting during the intermediate steps the signs of summation, which will be understood and taken up at the end, and remembering that for the cosine and sine, $f(D^2)=f(-n^2\omega^2)$ and $f(D'^2)=f(-\frac{s^2\pi^2}{l^2})$, we may now write,

$$u=\frac{HI_n}{kD+m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}\cos n\omega t\sin\frac{s\pi x}{l}.$$

Multiplying numerator and denominator by, $kD-m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)$, we have,

$$u=HI_n\frac{kD-m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}{k^2D^2-m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}\cos n\omega t\sin\frac{s\pi x}{l}.$$

Performing the indicated differentiation and substituting for D^2, its value,

$$u=HI_n\frac{-kn\omega\sin n\omega t\sin\frac{s\pi x}{l}-m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)\cos n\omega t\sin\frac{s\pi x}{l}}{-k^2n^2\omega^2-m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}$$

Since the signs of *all* the terms are now negative, we may write all of them positive and proceed to employ the same trigonometric transformation just previously explained. In this transformation, $kn\omega$ will correspond to "A", and $m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)$, to "$B$." Q will then be represented by,

$$\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}$$

and we may write for,

$$\frac{kn\omega}{\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}},\qquad \sin\epsilon$$

and for,

$$\frac{m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}{\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}}=\cos\epsilon,$$

where,

$$\epsilon=\tan^{-1}\frac{kn\omega}{m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}$$

Dividing both numerator and denominator of the last expression for u, by, $\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}$, and writing $\sin\epsilon$, $\cos\epsilon$, for the corresponding longer expressions, we have,

$$u=III_n\frac{\sin\epsilon\sin n\omega t\sin\frac{s\pi x}{l}+\cos\epsilon\cos n\omega t\sin\frac{s\pi x}{l}}{\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}}$$

Whence,

$$u=\sum_{n=1}^{n=\infty}\sum_{s=1}^{s=\infty}\frac{III_n}{\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}}\cos\left(n\omega t-\tan^{-1}\frac{kn\omega}{m\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}\right)\sin\frac{s\pi x}{l}.$$

(e)

$\tau_k=\frac{2\pi}{\sqrt{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}}$. Squaring and re-arranging, we have, $\frac{k^2}{4m^2}=\frac{s^2\pi^2a^2}{l^2}-\frac{4\pi^2}{\tau_k^2}$. Putting for the fundamental mode, $s=1$, and remembering that, $\tau_0=\frac{2l}{a}$, so that, $\frac{a^2}{l^2}=\frac{4}{\tau_0^2}$, we have $\frac{k^2}{4m^2}=\frac{4\pi^2}{\tau_0^2}-\frac{4\pi^2}{\tau_k^2}$, from which we see at once that, $\frac{k}{2m}=2\pi\sqrt{\frac{1}{\tau_0^2}-\frac{1}{\tau_k^2}}$. Mathematically this expression may have either a positive or a negative sign, but there can be no ambiguity physically for a positive exponent of "e" would represent a motion increasing without limit as time elapses and without the application of any force, which has no meaning.

(f)

For an explanation of the auxiliary equation and its use, see J. W. Mellor, Higher Mathematics for Students of Chemistry and Physics, third edition, p. 400. For its use in connection with the solution of (7), see Byerly, Fourier's Series etc., p. 113.

When the roots of the auxiliary equation are equal, trigonometric functions no longer appear in the solution.

A partial differential equation may be looked upon as equivalent to an infinite number of ordinary differential equations and there is a close formal analogy between the two types. It is often easiest to approach the solution by considering the form of solution of the corresponding ordinary equation. The solution of the ordinary equation with equal roots is of the form, $u = e^{ax}[A_1 + A_2x + A_3x^2 + \cdots\cdots]$ See Forsyth, Diff. Eq., p. 67. A_1, A_2 etc. are arbitrary constants whose value is determined by outside conditions not pertaining to the form of the equation itself. In the partial differential equation which is more general, we have arbitrary functions instead of arbitrary constants. In this particular equation functions of x may be taken to represent the infinite number of arbitrary constants which would be found in the infinity of ordinary differential equations, (one for every point of the string), which we might regard as the solution of the problem. (Compare Forsyth, Article 260, p. 512). Since the partial differential equation is linear, we shall expect no power of t beyond the first and may write as a tentative solution, $u = e^{\beta t}(F_1(x) + F_2(x)t)$ (I) in which we have to evaluate the functions $F_1(x)$ and $F_2(x)$ from the outside conditions. Here again we may profitably resort to analogy with the ordinary equation. The solution of the equation, $\frac{d^2u}{dt^2} + \frac{k}{m}\frac{du}{dt} + \frac{\mu}{m}u = 0$ (II) is, for the case of equal roots, $u = e^{\beta t}(A + B_t)$ (III). This might be regarded as representing the motion of some single point of the string, say the middle point, if it were entirely unaffected by the motion of other parts of the string. When t is zero, $u = U$, from which it is evident that $A = U$. Differentiating III, we have, $\frac{du}{dt} = \beta e^{\beta t}(A + Bt) + Be^{\beta t} \overset{t=0}{=} \beta A + B$ and this is zero when t is zero since it represents the velocity of the string, which is zero until it begins to move. Therefore $B = -\beta A$ or $B = -\beta U$. U is the maximum deflection of the single point whose motion is defined by (II) and in the partial differential equation the deflection of all of the infinite number of points which make up the string is repre-

sented by $F(x)$ when t is zero. U, then, corresponds to $F(x)$ and $U=A$ which corresponds to $F_1(x)$ in (I) so that $F_2(x)$ would correspond to $B=-\beta A=-\beta U=-\beta F(x)$. If we substitute these values in (I) our tentative solution becomes, $u=e^{-\beta t}[F(x)\cos\omega t+\frac{\alpha}{\omega}F_2(x)\sin\omega t]$ and on substituting for $F(x)$ a value, which satisfies the boundary conditions, we obtain immediately the equation (10) in the text. This, as there explained, satisfies the differential equation and the outside conditions and is therefore, an acceptable solution.

(g)

The amplitude term of the particular integral in (8) is,

$$\frac{HI_n}{\sqrt{k^2n^2\omega^2+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}}$$

Substituting for k, the value, $\frac{2s\pi am}{l}$ corresponding to critical damping of the sth. partial mode of the string's vibration, we have,

$$\frac{HI_n}{\sqrt{\frac{4s^2\pi^2a^2m^2n^2\omega^2}{l^2}+m^2\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)^2}}$$

Since m is a factor of both terms under the radical, we may take it out, and, performing the indicated involution of the second term, we have,

$$\frac{HI_n}{m\sqrt{\frac{4s^2\pi^2a^2n^2\omega^2}{l^2}+\frac{s^4\pi^4a^4}{l^4}-\frac{2s^2\pi^2a^2n^2\omega^2}{l^2}+n^4\omega^4}}$$

which reduces to,

$$\frac{HI_n}{m\sqrt{\frac{s^4\pi^4a^4}{l^4}+\frac{2s^2\pi^2a^2n^2\omega^2}{l^2}+n^4\omega^4}}$$

and since the expression under the radical is now a perfect square, we have

$$\frac{HI_n}{m\left(\frac{s^2\pi^2a^2}{l^2}+n^2\omega^2\right)}$$

Remembering that $a^2=T/m$, we may readily transform this expression to $\frac{HI_n}{\frac{s^2\pi^2T}{l^2}+mn^2\omega^2}$ and from this the form given in the text, equation (11), is obtained by multiplying numerator and denominator by l^2.

In the discussion of equation (11), the statement is made that if k and m were both zero the phase angle would be zero. The expres-

sion for the tangent of the phase angle can be put into better form to demonstrate that this statement is true. $\frac{2s\pi a n\omega}{l\left(\frac{s^2\pi^2a^2}{l^2}-n^2\omega^2\right)}$ $= \frac{2s\pi n\omega}{\frac{s^2\pi^2 a}{l}-\frac{n^2\omega^2 l}{a}}$. Remembering that $a=\sqrt{\frac{T}{m}}$ so that a is infinite when m is zero, we have, $(m=0)$, $\frac{2s\pi n\omega}{\frac{s^2\pi^2\infty}{l}-\frac{n^2\omega^2 l}{\infty}}$ which is manifestly equal to zero.

(h)

The solution of (7) for condition (c) given in equation (16) can also be derived as was that for condition (b) from the analogous solution of an ordinary differential equation. The solution of equation (II), appendix (f) for more than critical damping is, $u=C_1e^{\beta_1 t}+C_2e^{\beta_2 t}$ (I)h.

β_1 and β_2 are the two real and distinct roots of the auxiliary equation. For our present purpose we need not stop to determine the values of these roots, which would be similar in form to those of the corresponding partial differential equation. We are at present concerned with the determination of the arbitrary constants C_1 and C_2, with a view to obtaining a hint as to the probable form of the solution of (7) for the condition (c). Supposing as before, the ordinary differential equation to represent the motion of the middle point of the string entirely unaffected by the motion of other parts, we see that when $t=0$, $u=U=C_1+C_2$ (II) h.

Differentiating (I)h, and putting $t=0$, we have, since the velocity of the string is zero when $t=0$,

$$\frac{du}{dt}=\beta_1C_1e^0+\beta_2C_2e^0=0$$

$$\beta_1C_1=-\beta_2C_2 \qquad \text{(III)h}$$

From (II)h and (III)h we have,

$$U=C_1-\frac{\beta_1}{\beta_2}C_1=C_1\left(1-\frac{\beta_1}{\beta_2}\right)=C_1\left(\frac{\beta_2-\beta_1}{\beta_2}\right)$$

$$C_1=U\frac{\beta_2}{\beta_2-\beta_1} \qquad \text{(IV)}h$$

Also from (II)h and (III)h we have,

$$U=C_2-\frac{\beta_2}{\beta_1}C_2=C_2\left(1-\frac{\beta_2}{\beta_1}\right)=C_2\left(\frac{\beta_1-\beta_2}{\beta_1}\right)$$

$C_2 = U\left(\frac{\beta_1}{\beta_1 - \beta_2}\right)$ or for greater symmetry, we may write,

$$C_2 = -U\left(\frac{\beta_1}{\beta_2 - \beta_1}\right). \quad \text{(V h.}$$

The solution of (I)h, on substituting these values of C_1 and C_2, becomes,

$$u = U\left\{e^{\beta_2 t}\left(\frac{\beta_2}{\beta_2 - \beta_1}\right) - e^{\beta_1 t}\left(\frac{\beta_1}{\beta_2 - \beta_1}\right)\right\}$$

In the more general partial differential equation, U will be different for every value of x along the length of the string and will be zero at each end where the string is held fast. We must therefore replace U with some function of x which will define the law of variation of u along the string and in particular cause u to vanish when $x = 0$ and when $x = l$. The function which appears in the solutions for conditions (a) and (b) meets these requirements. Substituting it for U, we have at once equation (16) of the text. This satisfies the outside conditions and the differential equation and is therefore a satisfactory solution.

(i)

The formulas for these integrations are,

$$\int \lambda \sin\frac{s\pi\lambda}{l} d\lambda = -\frac{l\lambda}{s\pi}\cos\frac{s\pi\lambda}{l} + \frac{l^2}{s^2\pi^2}\sin\frac{s\pi\lambda}{l} \quad (1)$$

$$\int \lambda^2 \sin\frac{s\pi\lambda}{l} d\lambda = -\frac{l\lambda^2}{s\pi}\cos\frac{s\pi\lambda}{l} + \frac{2l^2\lambda}{s^2\pi^2}\sin\frac{s\pi\lambda}{l} + \frac{2l^3}{s^3\pi^3}\cos\frac{s\pi\lambda}{l} \quad (2)$$

The general reduction formula for obtaining the above is,

$$\int x^n \sin ax\, dx = -x^{n-1}\left(\frac{a x\cos ax - n\sin ax}{a^2}\right) - \frac{n(n-1)}{a^2}\int x^{n-2}\sin ax\, dx \quad (3)$$

The manner of deriving this formula by successive applications of the method of integration by parts can be found in Williamson's Integral Calculus, page 78, Article 65.

(j)

The same method of integration is employed as that referred to in appendix (i). Integration over a part only of the length of the string is more tedious, but not difficult. The following trigonometric relations were used in reducing the result.

$$\sin\frac{5s\pi}{12} = \cos\frac{s\pi}{12} \qquad \sin\frac{7s\pi}{12} = \cos\frac{s\pi}{12}$$

$$\cos\frac{5s\pi}{12} = \sin\frac{s\pi}{12} \qquad \cos\frac{7s\pi}{12} = -\sin\frac{s\pi}{12}$$

As a check on the correctness of signs etc. the integration was also performed from zero to $\frac{5l}{12}$ and from $\frac{7l}{12}$ to l and the sum of the results of the three integrations was found to be the same as that obtained in the much simpler single integration from zero to l.

$$\sqrt{\frac{1}{1-\frac{l^2k^2}{4m^2s^2\pi^2a^2}}} - \sqrt{\frac{4m^2s^2\pi^2a^2}{4m^2s^2\pi^2a^2-l^2k^2}} \quad (k)$$

$$= \sqrt{\frac{\frac{s^2\pi^2a^2}{l^2}}{\frac{s^2\pi^2a^2}{l^2}-\frac{k^2}{4m^2}}} = \sqrt{\frac{\frac{s^2\pi^2a^2}{l^2}}{\frac{\pi^2a^2}{l^2}(s^2-1)}} = s\sqrt{\frac{1}{s^2-1}}$$

In the cosine term, the expression under the radical becomes, $\frac{\pi^2a^2}{l^2}(s^2-1)$ and putting $\frac{2\pi}{\tau_0}$ for $\frac{\pi a}{l}$ we have, $\sqrt{\frac{4\pi^2}{\tau_0^2}(s^2-1)} = \frac{2\pi}{\tau_0}\sqrt{s^2-1}$.

The tangent of the phase angle becomes, $\frac{k}{2m}\cdot\frac{1}{\frac{2\pi}{\tau_0}\sqrt{s^2-1}} = \frac{1}{\sqrt{s^2-1}}$

Since, $\frac{k}{2m} = \frac{\pi a}{l} = \frac{2\pi}{\tau_0}$.

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March 1, 1924.

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The oral administration of insulin to rabbits.

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Two papers have recently appeared on the absorption of insulin in alcoholic solution from the stomach. Winter[1] stated that insulin in weak alcoholic solution administered to rabbits *per os* caused the blood sugar to fall to a low level. Blatherwick[2] concluded that the oral administration of a weak solution of alcohol to rabbits caused marked decrease in the blood sugar. There was no absorption of insulin from the stomach even though large amounts were given in alcohol, whether it was dissolved in ice water or in 0.4 per cent sodium carbonate solution. There was a hypoglycemia with insulin in alcohol and with alcohol alone.

Recently, with the assistance of Levin, Allen, Malich, Nachamie, Knox and Hoff, members of the second-year class in physiology, we have carried out a series of experiments on administration of insulin in dilute alcohol. The Shafer-Hartman method for the determination of the blood sugar was used.

The rabbits were given the alcohol daily in increasing doses, thereby becoming accustomed to the stomach tube and to the narcotic effect of the alcohol. The animals were not fed in the morning before or during the experiments. The diet consisted

* Introduced by H. B. Williams.

[1] Winter, L. B., *J. Physiol.*, 1923, lviii, 18.

[2] Blatherwick, N. R., Maxwell, L. C., and Long, M. L., *Am. J. Physiol.*, 1924, lxvii, 346.

TABLE I.

Date 1924	Rabbit No.	Wt.	Before injection	½ hr. after	¾ hr. after	1 hr. after	1¼ hr. after	1½ hr. after	1¾ hr. after	
			Glucose, mg. per 100 cc.							
Jan. 8	97	2400	130							1 cc. 95 per cent alcohol in 25 cc. H_2O. One additional cc. of 95 per cent alcohol was given each succeeding day in 20 cc. distilled H_2O, making 5 cc. on Jan. 12th.
Jan. 12	97	2400	158		154			111		5 cc. 95 per cent alcohol in 20 cc. H_2O.
15	97	2400	162	131						5 cc. 95 per cent alcohol in 20 cc. H_2O.
19*	97	2500	163	165			216	184		5 units of insulin in 21 cc. 20 per cent alcohol.
22	97	2500	160				136		135	100 units of insulin in 20 cc. 20 per cent alcohol (slight amount spilled).
7	56	2270	112							1 cc. 95 per cent alcohol in 25 cc. H_2O.
11	56	2300	122							One additional cc. of 95 per cent alcohol was given each succeeding day in 20 cc. distilled H_2O making 5 cc. on Jan. 14th.
14	56	2300	124	132		138		160		25 cc. of 20 per cent alcohol.
18	56	2515	116	148		160		170		21½ units of insulin in 20 cc. of 20 per cent alcohol.
20	56	2610	128	160		128				50 units of insulin in 20 cc. of 20 per cent alcohol.
Feb. 1	56	2600	154	138		108		98		0.89 units injected subcutaneously.
1	97	2500	166	77		82		84		0.875 units injected subcutaneously.

*Rabbit struggled.

of oats, hay and cabbage[3], a diet which should have rendered the rabbits sensitive to insulin[4].

The insulin used was the "Lilly U-20 units."

The results of these experiments are exhibited in the accompanying table.

CONCLUSION

While the results of our work are not conclusive, they make it seem improbable that insulin when administered in alcoholic solution is absorbed from the stomach in amounts that would give this method of administration any practical importance.

My thanks are due to Prof. E. L. Scott for suggesting this work.

[3] Sherman, H. C., and Gettler, A. O., *J. Biol. Chem.*, 1912, xi, 323.

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The Future of Biological Investigation*

By Horatio B. Williams, Dalton Professor of Physiology, College of Physicians and Surgeons, Columbia University.

IN choosing a topic for these brief remarks it seemed to me most appropriate to select one which would permit me to touch lightly certain aspects of the educational problem which concerns us all. I wonder what your views are regarding the value of education. Perhaps in common with many you think most of its utilitarian values. I think the average person regards an education as a training which will fit him the better to perform his daily tasks and enable him the more easily to outstrip his fellows in the struggle for material success. It is true that education will do this, but is this all? What after all is this material success for which so many people give their best efforts? To the lower animals and, I fear, to many men material success means meeting the needs of the body for food and shelter for today so that its existence may be continued until tomorrow and so on in endless repetition until at last the law of the sequence is broken and the individual's struggle for continued existence is at an end. Rightly regarded, the main purpose of an education is not grossly utilitarian. The capacity to derive more than mere animal comfort from material success depends upon the development of those powers which it is the function of education to cultivate. From this standpoint all education is good, all knowledge is a source of joy to its possessor, but for the cultivation of a serene outlook on life and its vicissitudes, for the maintenance of a sound balance and correct perspective the discipline of Medicince seems especially fortunate.

During the early years of childhood our every need is anticipated and we receive constant care. Later we must learn to depend on our own efforts and so gradually this parental care must be relaxed so that we may learn self-dependence. So in our education at first we are merely given facts to learn. How anyone came to know them as facts need not for the present trouble us. Eventually we must depart from this method, for in time we shall reach a stage when the answers to our questions are not to be found in books nor obtained from men, but must be obtained direct from Nature. Some of you, I hope most of you, have already begun to reach the stage of intellectual independence, but it has been our observation in the past that the medical student craves definite statements of fact such as he becomes accustomed to in his more elementary studies. In some branches of the work this craving can be gratified to a limited extent, but in the greater number of cases definite dogmatic statements would be as likely to mislead as not. The medical sciences are growing. Methods of investigation improve. Things which were regarded as facts of observation a few years ago are shown by more accurate measurements not to be so. Theories particularly are subject to change in the light of new knowledge. You will not infrequently find several theories presented to account for some biological process. The evidence for and against these will be given and the decision left to you. You may find the decision hard to make and wish your instructor would make it for you. This he must not do, for in the future as knowledge advances you must be prepared to make your own decisions, and this you can learn to do only by beginning. The work of the physician and surgeon calls for ability to assume individual responsibility and make quick decisions in matters of great moment. You must begin doing this in your training

*Address delivered at the opening exercises of the College of Physicians and Surgeons, Columbia University, on September 23, 1925.

period. If all of the fundamental medical sciences were fully understood, if disease always manifested itself in a constant and definite way, if there were for each ailment a definite curative treatment or surgical procedure, the teaching would be easier, but I doubt whether the subject would make the same appeal it now does to the man of intellectual courage.

There was a time when men believed that all life processes were so essentially mysterious, so inextricably bound up with the "vital" forces supposed to be different from those obtaining in the world of non-living things that any attempt to understand the mode of operation of the living organism must be doomed to disappointment from the beginning. The success which attended Harvey's investigations on the circulation of the blood is a brilliant example of the fruitfulness of common sense and correct mechanical ideas applied to a biological problem. I presume that Harvey's teleological reasonings would provoke a smile nowadays, but that is largely a habit of thought and manner of expression. The point to be emphasized is that instead of allowing himself to be awed into inaction by the great mystery of life he proposed attempting to apply to one of the mysteries the mechanical principles which hold in non-living mechanisms—and he found that the plan worked.

In the early days of chemical inquiry those substances which were derived from the life processes of the plants and animals were called "organic" chemicals and it was believed that they could be produced only by the activities of plant or animal organs. To this day we preserve a memorial of that stage of thought in that we denominate a large branch of chemistry "Organic Chemistry." When, less than a century ago, Woehler succeeded in preparing from inorganic chemicals urea, a substance hitherto known only as a product of animal metabolism, it was realized that there is no necessary reason to assume other than the usual laws of chemistry to account for the production of certain at least of the chemical substances associated with life processes. To enumerate all of the substances which are the natural product of plant or animal life that have now been synthesized by the chemist from non-living materials would be no inconsiderable task.

Johannes Mueller, leading physiologist of the early part of the last century and teacher of DuBois-Reymond and Helmholtz, held the view that it would be impossible to measure the rate of transmission of the nervous impulse. He believed the processes of thought showed that transmission must be instantaneous. A few years later his own pupil, Helmholtz, measured the rate with simple contrivances and showed that in the sciatic nerve of the frog it is not only finite, but of the same order of magnitude as the speed of a not very fast railroad train.

With the discovery of oxygen and its relation to combustion it was not long before the suspicion was aroused that oxidation might have something to do with animal heat. The expired air was found to be richer in carbon dioxide and poorer in oxygen than the inspired. Men had become familiar with the steam engine as a means of converting the chemical energy of coal into mechanical work. Could it be the oxidation of foodstuffs or of body substances elaborated from the foodstuffs which furnished animal heat and caused muscular movements? I have not time to go into the history of this matter and shall pass over the fundamental researches of Voit and Pettenkofer to mention briefly the culmination of these investigations in the work of Atwater and Rosa with the respiration calorimeter. These investigators proved beyond peradventure that the law of conservation of energy extends to the animal body. They showed that the burning of a gram of foodstuff outside the body yields precisely the same amount of energy as its slow oxidation within the body.

One might continue multiplying instances of this kind, but those mentioned are sufficient in number, variety and importance to illustrate the point I wish to bring out. While it has not been proven that there are no special "vital" forces, the greatest progress in understanding the activities of living things has invariably resulted from attempts to extend to the living organism those physical and chemical conceptions which have been found true in the non-living world. The assumption of a mysterious "vital" agency not subject to in-

vestigation by the methods of physics and chemistry tends from the beginning to deaden thought and kill initiative. I have no quarrel with the man who feels that such human qualities as the sense of honor, of altruism, of patriotism are too refined to admit of explanation as conditioned reflexes. It is not necessary for our purpose to assume at the outset that *all* life processes are of a mechanistic nature controlled by the same laws as those which obtain in non-living matter. Such an assumption in the present state of our knowledge would be quite as difficult to defend as the vitalist hypothesis. All that seems requisite to put the mind in the proper attitude for investigation is to determine that we shall endeavor to ascertain how far we may explain and account for the phenomena of life in terms of the laws which have been found to apply to physical and chemical processes in the inanimate world.

In presuming to choose for the title of my address this morning "The Future of Investigation in Biology," I had not the temerity to think of trying to predict what the future will or will not bring in the way of particular results or even how, precisely, the result of increasing our biological knowledge is to be obtained. It is rather my purpose to indicate in a general way what seems to be requisite in order that some such fundamental advance may be made in biology as was that which Newton made in mechanics when he was able to prove the validity of his extension of the law of falling bodies on the earth to bodies throughout all space as far as data are available. I presume everyone has heard the story of Newton and the apple which fell on his head. It is hardly necessary to say that it is apocryphal. The true history is less well known and as the essentials can be given in a few words and bear upon what follows I may be pardoned for a slight digression. It occurred to Newton that perhaps the attractive force which causes bodies near the earth to gravitate toward it might extend into space indefinitely and if so even as far as the moon, perhaps accounting for her retention in her orbit. He immediately made a calculation to determine how far a body would fall in a minute at the distance of the moon if its fall were due to the same force which attracts bodies at the earth's surface. He found that the distance per minute would be something over fifteen feet, whereas the actual deflection of the moon from the direction of the tangent to her orbit was about thirteen feet per minute. I fear many of us would have regarded that as a rather close approximation, but Newton found it so unsatisfactory that he at once abandoned the theory. Thirteen years later he was again led to consider this theory and became aware of a better determination of the value of the earth's mean radius which had been made since his first calculation. The value he had used in 1666 had led to a wrong estimate of the moon's distance. With the new value he obtained a correspondence between theory and measurement which proved satisfactory to him. So satisfactory has it since proved that with ever increasing accuracy of observation and with data available not alone from our own solar system but from distant double stars, Newton's generalization is found to fit the facts. Only within recent years and in the fields of light and electricity have facts been observed which seem to require some slight modification of the Newtonian mechanical conceptions. Biology today is in something like the state in which mechanics and astronomy were in the days of Hipparchus or Ptolemy. We are living in the age of the epicycles. Our greatest need is for some generalization which will reduce to order the tremendous chaos of observations we now have before us. The material from which the generalization must come is the body of observed facts. To be able to generalize from these facts, however, it is necessary that someone shall be familiar with all the different types of observational data which may reveal something essential to the generalization. It will also be essential that we have a fair general knowledge of the basic principles of chemistry and physics and some facility in applied mathematics. At first sight this would seem to require an incredible amount of preparation. Probably it would in the present state of biological science. No great advance is ever made by the unaided effort of one man. Many contributed their share before Newton was able to take the step he did. Many have contributed already in biology, but the

kind of contribution we need now is a summarizing of the data. We are all of us at present too close to the great mass of detailed observation to see its broader implications. We need not waste time regretting this. The work which has been done had to be done, or at least much of it had to be done and possibly much more of the same type of work will still have to be done before biology is ready for a simplifying generalization. The broader view which is denied to us will be the heritage of our successors. They cannot, must not spend their time delving into the great mass of details. Gradually, through the efforts of many workers the more essential phenomena will be separated from the incidental so that it will be possible for one individual to survey the field without devoting so much of his lifetime to the task as to leave no opportunity for that familiarity with the related sciences which is requisite before a generalization can be made. Meanwhile we should do our best to secure *reliable* data so that the "Newton of Biology," to use Cuvier's expression, may not be baffled in his work as Newton was by attempting to use inaccurate information. I believe that one of the most urgent needs in biological work today is a study of this very matter of the reliability of conclusions drawn from biological experimentation.

In so far as biological phenomena rest on a physical or chemical basis, we may hope for assistance from the advances made in these sister sciences. We do not know what day an important discovery or generalization in chemistry or physics may permit the coordination and understanding of many seemingly unrelated biological phenomena. If we could learn to express our biological facts without the use of quite so much special terminology, it is even conceivable that men in other fields of science might contribute to its theoretical handling. I think we might well give attention to this matter of unnecessary technical terminology.

We may perhaps draw a lesson from one of the features which has characterized the development of chemistry in recent years. Although scientific chemistry dates from the introduction of quantitative methods, the application of mathematical analysis with a view to pushing chemical knowledge beyond the bounds of observation is comparatively recent. Thirty years ago chemistry might have been described as a non-mathematical science. To the man who has not at least an elementary knowledge of mathematical analysis modern chemistry is a closed book. In fact the application of mathematical methods promises to do as much for chemistry as it has done for physics. In most branches of biological work it is difficult at present to secure quantitative accuracy comparable with that attained by the pure chemist or physicist. Gradually as our methods improve it should be possible to investigate our results under the microscope of mathematical analysis. When this can be done we may begin to hope for the long-awaited generalization which is to bring order out of chaos, reduce the size of our volumes and give us time for a broader outlook.

What should be the preparation of the biologist of the future? I can attempt to answer this question only in general terms. Medicine, in its practical aspects, is applied biology, and the preparation for its study will depend on the character of the biology of the time. It must of necessity be along rather definitely prescribed lines. For the student of pure biology or for the medical student who determines for the first time that he is to devote his life to investigation and teaching rather than to practise more latitude might be allowed. It seems to me most desirable that there should come into the biological sciences men of widely different training. There should be some who are trained as chemists, others trained as physicists with the usual mathematical equipment of the physicist. Still others should probably have much the type of training which most of our present day biologists have had, with perhaps somewhat more emphasis on precise quantitative methods.

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STUDIES ON THE ULTRAFILTRATION AND ELECTRODIALYSIS OF INSULIN SOLUTIONS

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The methods used thus far for the purification of insulin may be divided into two classes; fractional precipitation of the protein-like substances by regulation of the hydrogen ion concentration, and precipitation by various reagents. Among the first type may be noted the methods of Doisy, Somogyi and Shaffer (1923), (1924), and among the second, those of Collip (1924), Dudley (1923), Dudley and Starling (1924) Moloney and Findlay (1923), Best and Scott (1923), Shoule and Wahlo (1924), and N. B. Fischer (1923). The only departure from either of the above methods is the meager and rather incomplete work of Locke and Hirsch (1924a), (1924b) on electrodialysis.

In this investigation the purpose was to find a method for separating or concentrating the active constituent in insulin solutions that would avoid the addition of precipitants, absorbants and buffers now commonly used. It was the aim of the authors to obtain the active material simply and quickly in sufficient purity for chemical investigation.

Successful application of ultrafiltration through graded membranes, and of methods of electrophoresis and electrodialysis to the separation of amyloses in starch were lately carried on in this laboratory (Taylor and Iddles, Dissertation, Columbia, 1925). These methods suggested themselves in attempting to purify insulin solutions.

Although the use of the first method did not give the desired result in the way of purification, nevertheless it did bring out some interesting properties of insulin. For this reason the technique has been given in some detail. On the other hand, the second method lent itself nicely to concentration of the physiologically active substance.

EXPERIMENTAL WORK. A. *The ultrafiltration of insulin solutions through collodion membranes of varying permeability.* The insulin solu-

[1] The authors wish to express their thanks to Dr. G. H. A. Clowes, through whose courtesy the Eli Lilly Company supplied insulin for this investigation.

tions used were the clinical U-20 iletin (Insulin-Lilly) (1923). An apparatus, employing membranes, made and graded according to Nelson and Morgan (1923–24), served to give all the data herein recorded. The plates, on which the membranes were made, were floated on mercury to insure perfect leveling, the only departure from the Nelson and Morgan technique.

The ultrafiltration unit consisted of the parts, shown assembled and correspondingly lettered in figure 1.

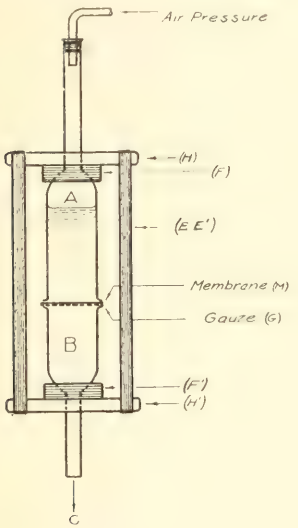

Fig. 1

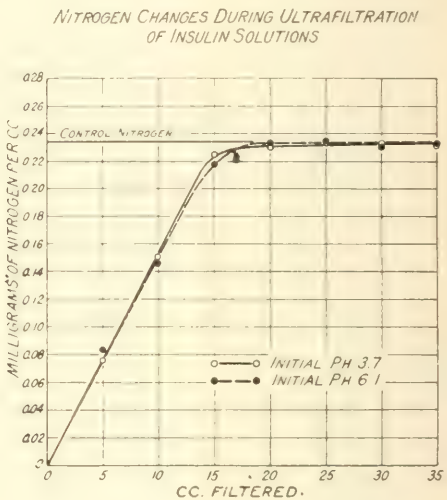

Fig. 2

Fig. 1. *A*, Gooch funnel serving as an insulin compartment; *B*, Gooch funnel serving as the lower half of the ultra-filter; *C*, a receiving vessel for the filtrate; *M*, a permeable membrane; *G*, a nichrome gauze to support the membrane and prevent its rupture during filtration; *EE'*, elastic bands to hold the upper and lower funnels rigid; *HH'*, wooden collars, fitted on the funnels, over which the bands *EE'* were stretched; *FF'* felt buffers to prevent fracture of the glass due to the pressure exerted by the bands, *EE'*.

A tight seal between the upper and lower funnels, and the membrane was effected by means of a rubber-paraffin cement.

When the apparatus had been tested and found to be leak-proof, an insulin solution, usually 15 cc., was placed in the upper part *A*, and a receiving vessel was placed in position *C*. Air, freed from carbon dioxide by passage through soda lime, and kept under a constant pressure of 60 cm. of water by means of a manostat, was then applied to the upper part of the apparatus and the preliminary filtration was begun. When the filtrate gave a distinct precipitate with phosphotungstic acid, the filtration was stopped.

The necessity for this preliminary filtration and test with phosphotungstic acid became apparent from some introductory experiments on ultrafiltration. These showed that before any conclusions could be drawn regarding the permeability of collodion membranes to insulin as measured by the physiological activity of the filtrates, it was essential first to saturate the membranes to be used with insulin. This was accomplished best by the preliminary filtration. The saturation of the membrane could be determined easily by the formation of a white precipitate when a solution of phosphotungstic acid (Sherman, 1912) was added to the filtrate. The absence of a phosphotungstic acid precipitate indicated that no active principle had passed into the filtrate, usually because the membrane had not become saturated. The proof that a filtrate giving no precipitate with phosphotungstic acid is physiologically inactive is shown by the following experiment:

Five cubic centimeters of clinical U-20 insulin were ultrafiltered through a grade 15.7 membrane, which had not been saturated previously by a preliminary filtration. The filtrate gave no precipitate with phosphotungstic acid while the control sample gave a copious one under the same treatment. Samples of filtrate and control were tested biologically for blood sugar reducing properties.

The physiological activity in these samples and in all samples mentioned in subsequent work was tested out on rabbits weighing about 2 kilos. The standard injection per animal per kilo of body weight was one physiological insulin unit, which has been defined by the Toronto Insulin Committee (1923) as, "one-third of the amount required to lower the blood sugar below 0.045 per cent and cause convulsions in a rabbit weighing 2 kilograms, which has been previously starved for 24 hours, within 2 to 5 hours after injection." Three samples of blood were drawn from each animal, an initial sample, one $\frac{3}{4}$ hour after injection, and one $1\frac{1}{2}$ hours after injection. The sugar in each sample was quantitatively determined by the Shaffer and Hartmann (1921) method.

The results of these physiological tests on the filtrate and control sample are given in table 1.

From the above data, it can be seen readily that the filtrate which gave no precipitate with phosphotungstic acid, was totally inactive, while the control, which gave a copious precipitate was active.

I. Effect of ultrafiltration on physiological activity. In all subsequent experiments, the membranes were saturated with insulin by a preliminary filtration, and then washed three times with 10 cc. of distilled water. A fresh insulin solution then was placed in part *A*, a clean receiving vessel at *C*, and the regular filtration was begun.

When about one-half of the insulin solution had passed through the through the membrane, the filtration was stopped. Samples of control,

TABLE 1

GRADE OF MEMBRANE USED	DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER $1\frac{1}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP AFTER $1\frac{1}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
15.7	Control	135	74	80	61	55	Clinical U-20 insulin gave good precipitate with phosphotungstic acid
15.7	Control	141	66	68	75	73	
15.7	Control	130	68	74	62	56	
Average		135	69	74	66	61	
15 7	Filtrate	110	108	110	2	0	Gave no precipitate with phosphotungstic acid
15 7	Filtrate	122	118	118	4	4	
15 7	Filtrate	124	128	128	0	0	
15 7	Filtrate	122	128	122	0	0	
15 7	Filtrate	108	128	128	0	0	
15 7	Filtrate	105	118	114	0	0	
Average		115	121	120	0	0	

filtrate and remainder on the membrane, were tested for physiological activity as described above.

This experiment was repeated for several membranes of varying grades of permeability with the hope of obtaining some fractionation of the active from the inactive material, as would be evidenced by increased activity of the solution above or below the membrane. Table 2 gives the results obtained.

From the above data, which represent averages of series of animal results on each sample tested, it is seen that within the experimental error, the average sugar drop per unit injected is the same for the control, filtrate and remaining solution. It may be concluded, then, that the active principle of insulin preparations can be ultrafiltered through collodion membranes of varying permeability without loss of physiological activity, and further, that no apparent fractionation takes place, during the ultrafiltration.

II. Nitrogen changes during ultrafiltration of insulin solutions. The practicability of ultrafiltration of insulin solutions through graded membranes without loss of physiological activity having been shown, it was decided next to study the nitrogen content of the filtrate, with the view of ascertaining whether nitrogen could be removed from insulin by ultrafiltration without impairing its activity. This could be accomplished best, by collecting fractions of the filtrate and determining the nitrogen content and physiological activity in each.

The ultrafiltration apparatus and membrane technique used was the same as described in the previous section, with the exception that each successive five cubic centimeters of filtrate were collected in separate vessels, and the membrane was not saturated previously. The nitrogen content in each fraction was then quantitatively determined by the micro-Kjeldahl technique of Pregl (1923), and each fraction was tested for precipitation with phosphotungstic acid. This was done prior to testing the fraction for physiological activity, because as has been mentioned previously, the absence of a phosphotungstic acid precipitate indicates the absence of activity in the filtrate.

Several fractional ultrafiltration experiments were carried out with membranes of different degrees of permeability ranging from 2.0 to 12.0, (graded according to Nelson and Morgan (1923-24)) and with insulin solutions on both the acid and alkaline sides of pH = 4.8 (the recorded isoelectric point of insulin (Council on Pharmacy and Chemistry 1923)). The results obtained in all these investigations are shown by the detailed data from two experiments, both on the same grade membranes but on different sides of the isoelectric point. Concurrently, a colorimetric determination of the hydrogen ion (Clark, 1924) concentration on each fraction was made. The data obtained have been recorded in table 3 and are shown graphically in figures 2 and 3.

TABLE 2

GRADE OF MEMBRANE USED	DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A – B) BLOOD SUGAR DROP AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A – C) BLOOD SUGAR DROP AFTER $1\frac{3}{4}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
20.7	Control	137	61	61	76	76	Clinical U-20 insulin 1 unit per kilo was injected
12.0	Control	122	63	73	59	49	
11.5	Control	135	69	71	66	64	
Average		131	64	68	67	63	
20.7	Filtrate	127	75	59	52	68	Filtrate from U-20 insulin through membranes whose grade is given in first column
19.6	Filtrate	124	69	65	55	59	
12.0	Filtrate	121	68	64	53	57	
12.0	Filtrate	120	66	56	54	64	
11.5	Filtrate	134	64	62	70	72	
3.6	Filtrate	134	71	53	63	81	
Average		127	69	60	58	67	
20.7	Remainder	110	64	57	46	53	Solution on membrane when filtration was stopped
19.6	Remainder	136	74	62	62	74	
12.0	Remainder	115	52	41	63	74	
11.5	Remainder	123	59	56	64	67	
3.6	Remainder	128	69	52	59	76	
Average		122	63	53	59	69	

Figures are averages for:

3 rabbits on samples: Control 20.7, 12.0, 11.5. Filtrate 20.7. Remainder 20.7.

4 rabbits on samples: Filtrate 3.6. Remainder 3.6.

5 rabbits on samples: Filtrate 12.0.

6 rabbits on samples: Filtrate 19.6, 12.0, 11.5. Remainder 19.6, 12.0, 11.5.

From the above results it is apparent that a low nitrogen content of the filtrate, probably caused by loss in saturating the membrane, is accompanied by an equivalent drop in physiological activity.

Since collodion membranes removed hydrogen ion from solutions (Bethe and Toropoff, 1914, 1915), it is to be expected that the insulin solution above the membrane should become progressively more alkaline as the ultrafiltration continues. As soon as a pH of about 4.8 was reached, precipitation of insulin started. On the alkaline side of the isoelectric point, this change in hydrogen ion did not take place and the precipitation of insulin on the membrane was avoided.

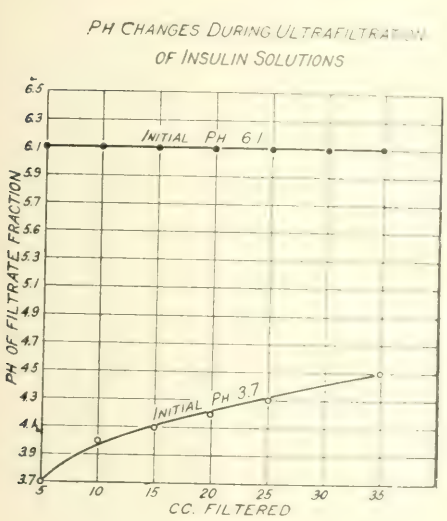

Fig. 3

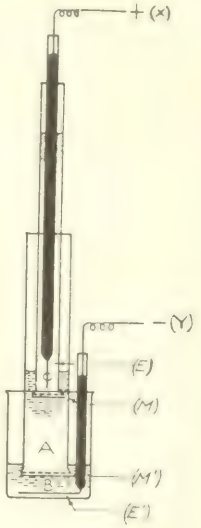

Fig. 4

Fig. 4. *A*, an insulin compartment filled with about 25 cc. of U-20 insulin (Lot 10); *BC*, electrode compartments filled with 20 cc. of distilled water; *EE'*, the platinum electrodes; *MM'*, impermeable membranes.

B. The electrodialysis of insulin solutions. *1. The formation of an active sediment by electrodialysis of insulin solutions.* The inactivation of insulin by both oxidation and reduction (Shonle and Waldo, 1923), demanded the use of a special type of electrolytic cell for the investigation of the behavior of the active principle when subjected to electrodialysis. The essential feature in the design of the cell must be, therefore, complete removal of the electrodes from the insulin solution undergoing electrodialysis. This would help prevent oxidation or reduction of the active principle by any secondary products of the electrolysis, formed at the electrodes.

The electrolytic cell: The cell finally adopted and used in all experi-

TABLE 3

PART A

Grade membrane used = 10.0.
Initial pH = 3.7 (acid side of isoelectric point).

DESCRIPTION OF MATERIAL	A	B	C	D; (A − B)	E; (A − C)	NITROGEN CONTENT IN MILLIGRAMS N. PER CUBIC CENTIMETER	pH OF FILTRATE FRACTION	REMARKS
	INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC.	BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.	BLOOD SUGAR DROP AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	BLOOD SUGAR DROP AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.			
Control	117	52	65	65	52	0.234	3.7	Clinical U-20 insulin
Filtrate fraction no. 1						0.076	3.7	No precipitate with phosphotungstic acid
Filtrate fraction no. 2						0.151	4.0	Very slight precipitate with phosphotungstic acid
Filtrate fraction no. 3						0.225	4.1	Some precipitate with phosphotungstic acid
Filtrate fraction no. 4						0.230	4.2	
Filtrate fraction no. 5						0.233	4.3	Heavy precipitate with phosphotungstic acid
Filtrate fraction no. 6	143	81	70	67	73	0.233		Volume too small for pH
Filtrate fraction no. 7						0.231	4.5	

PART B

Initial pH = 6.1 (alkaline side of isoelectric point).

DESCRIPTION OF MATERIAL	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC.	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.	D; (A − B) BLOOD SUGAR DROP AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC.	E; (A − C) BLOOD SUGAR DROP AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC.	NITROGEN CONTENT IN MILLIGRAMS N_2 PER CUBIC CENTIMETER	pH OF FILTRATE FRACTION	REMARKS
Control	126	86	67	40	59	0.234	6.1	U-20 insulin adjusted to pH 6.1
Filtrate fraction no. 1						0.084	6.1	No precipitate with phosphotungstic acid
Filtrate fraction no. 2						0.146	6.1	Very slight precipitate with phosphotungstic acid
Filtrate fraction no. 3						0.218	6.1	Precipitate with phosphotungstic acid
Filtrate fraction no. 4						0.233	6.1	
Fraction no. 5	122	64	62	58	60	0.235	6.1	Heavy precipitate with phosphotungstic acid
Filtrate fraction no. 6						0.231	6.1	
Filtrate fraction no. 7	135	60	49	75	86	0.233	6.1	2 rabbits took convulsions

ments on electrodialysis, was composed of the parts shown assembled and correspondingly lettered in figure 4.

The insulin compartment, A, and electrode compartment, C, were sealed off by means of collodion membranes, MM', made in such a manner as to render them impermeable to the active principle but permeable to electrolytes during the electrodialysis.

The electrodialysis membranes: The membranes, MM' mentioned above, were prepared by the following method. The end of the cell unit upon which the membrane was to be made, was covered with a tightly stretched piece of cheese-cloth, (shown as dotted lines in fig. 4) which served as a framework for the membrane. The covered end of the unit was then dipped into a fairly pure collodion solution (Merk's collodion), removed, and revolved for ten minutes. At the end of this time all of the ether and most of the alcohol had evaporated, leaving on the cloth gauze a rather impermeable membrane. This was repeated three times, thereby insuring against leaks, and giving a very impermeable membrane. These membranes then were soaked in distilled water for at least five hours before being used. They did not "blush" in contact with water but remained perfectly transparent.

The electrodialysis technique: When the cell had been set up as shown in figure 4, the insulin solution[2] to be electrodialysed was placed in compartment A, the proper volume (20 cc.) of distilled water in compartments B and C, and the current turned on. A potential of 100 volts was applied between x and y by means of direct current from the lighting service, passing through suitable resistance. With this drop in potential across x and y, a current of 5 milliamperes flowed through the cell at the outset. In all subsequent experiments the polarity of the cell, unless otherwise stated, was that shown in figure 4, with C the anode and B the cathode compartment.

Current was passed for any desired period of time. The circuit was then broken, compartment A was disengaged from the cell, and the liquid decanted from the sediment which had been formed on the membrane M during the electrodialysis.

The sediment was washed into a small Büchner funnel with absolute methyl alcohol. The residue on the filter was washed several times with absolute alcohol and finally with absolute ether. This last treatment is not absolutely essential, but it produces an almost white sediment instead of a dark brown one and materially increases the ease with which the substance can be removed from the paper. The sediment was removed from the filter and dried in vacuo at 30°C. to constant weight.

[2] More concentrated solutions of insulin were diluted until they were equivalent to U20 (Lilly). This contains approximately 2 mgm. solids per cubic centimeter of which from the present work about 1 mgm. appears to be physiologically active.

Since insulin is reported to become inactivated when heated above 30°C. (Best and Macleod, 1923; Doisy, Somogyi and Shaffer 1923; Dudley 1924; Murlin, Piper and Allen, 1923) none of the samples was heated higher than that temperature. All subsequent data are reported on constant weight at 30°C. in vacuo.

II. The physiological activity of the sediment. The physiological activity of the sediment produced by the electrodialysis of insulin solutions was tested, in order to determine the effect of the electric current on the potency of the active principle.

The dried sediment was weighed out in 2 to 2.5 mgm. samples, and dissolved in such a volume of physiological salt solution (0.9 per cent sodium chloride solution) acidified with 4 drops of N 10 hydrochloric acid per 2 cc. of solution, as would give a concentration of 1 mgm. per cubic centimeter. Then $\frac{1}{2}$ cc. of this solution was diluted to 10 cc. with physiological salt solution, giving a final concentration of 0.05 mgm. per cubic centimeter of solution. The standard injection was 1 cc. of this diluted solution or 0.05 mgm. per kilo of body weight. Control samples were made up in exactly the same manner to contain about 0.1 mgm. per cubic centimeter of final solution, and were injected at about 0.1 mgm. per kilo of body weight. The animal technique and blood sugar determinations were carried out as described under ultrafiltration. Several samples of sediment prepared from different batches of insulin, together with adequate controls, were tested for physiological activity as described, and the results obtained have been recorded in table 4.

From the data given in table 4, it can be seen that the average blood sugar drop for $\frac{3}{4}$ hour and $1\frac{3}{4}$ hours after injection, on 7 control samples, was 61 and 67, while the average on 10 comparable sediment samples for the same periods of time, was 66 and 68 mgm. per 100 cc. of blood. The average activities were about equal. From table 4 it also will be noted that the unit weight of solid material injected per kilo was not equal for the control and sediment samples, the latter being only 0.05 mgm. as compared to 0.1 mgm. of the former, or one-half as much. Since half the unit weight of sediment produces about the same lowering of blood sugar in the experimental animals, as a whole unit weight of original insulin, it has been concluded that the sediment produced by the electrodialysis of insulin solutions is apparently twice as active, physiologically, as the original material. As will be shown later, these results take into consideration the experimental errors.

III. The physiological activity of the filtrate. The greater activity of the sediment could be explained best by assuming that some relatively inactive substance was being removed from the active material during the electrolysis of insulin solutions, or in other words, the original material was being fractionated in some way into two substances, one of high acti-

TABLE 4

EXPERIMENT NUMBER	DESCRIPTION OF MATERIAL	MILLIGRAMS PER KILO INJECTED	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
1 and 2	Control	0.12	120	58	59	62	61	Clinical U-20 iletin (Insulin-Lilly)
3	Control	0.10	129	81	63	48	66	
4 and 5	Control	0.13	124	65	60	59	64	
7 and 8	Control	0.10	134	74	57	60	77	
9 and 10	Control	0.12	129	77	77	52	52	
15	Control	0.10	135	67	58	68	77	
12, 13, 16	Control	0.10	144	67	71	77	73	Concentrated non-clinical iletin. diluted to U-20 concentrated
Average			131	70	65	61	67	
1	Sediment	0.07	132	120	119	12	13	Insulin in contact with electrodes
3	Sediment	0.05	125	57	61	68	64	First active sample
4	Sediment	0.05	127	56	42	71	85	
5	Sediment	0.05	150	110	96	40	54	Precipitated from (CH_3OH—HCl) by dry ether
7	Sediment	0.05	142	74	63	68	79	Formed at pH 3.6—
8	Sediment	0.05	121	60	63	61	58	Formed in ½ hour at pH 3.6 3.7.
9	Sediment	0.05	135	74	69	61	66	From clinical U-20 iletin
10	Sediment	0.05	144	58	77	86	67	
15	Sediment	0.05	133	79	68	54	65	From clinical U-20 iletin
12	Sediment	0.05	127	72	77	55	50	From concentrated non-clinical iletin
13	Sediment	0.05	127	70	69	57	58	
16	Sediment	0.05	149	71	63	78	86	
Averages on nos. 3, 4, 7, 8, 9, 10, 15, 12, 13, 16			133	67	65	66	68	

Note: Figures above are averages on data from 3 to 4 rabbits per sample tested.

ity, and one of low activity. The validity of this assumption could be shown by the isolation of a relatively inactive product from the filtrate remaining after the removal of the active sediment.

This was attempted, and an organic substance showing little physiological activity was obtained from the filtrate by two different procedures. These consisted of *a*, evaporation of the clear filtrate from the electrodialysis to constant weight at 30° C. in vacuo; and *b*, adjustment of the pH to 4.8, filtration of the precipitate formed and drying the latter in vacuo as usual. Method *a* gave a substance which showed greater activity than the material produced by *b* as can be seen from the physiological results on filtrate samples recorded in table 5.

The average drop of sugar per 100 cc of blood for the six filtrates was 28 and 15, as compared to 66 and 68 mgm. for the precipitates (table 4) for the same time intervals after injection, thereby showing that the physiological activity of the organic substance remaining in the filtrate after removal of the active material is less than half that of the sediment. Even though these figures do not represent an absolute cleavage of the original insulin into an active and inactive substance, which would be a very difficult accomplishment as shown by some of the work presented in following sections, nevertheless they do indicate that the production by electrodialysis of a substance of increased activity is due to fractionation of the original material.

IV. The deposition of the active sediment. The deposition of an active sediment during the electrodialysis of insulin solutions had been established, so far, but no change either of the polarity of the cell or of the initial pH of the solution had been attempted. It was of interest to investigate both of these changes from the point of view of determining whether the active material always deposited at the same electrode, or migrated in different directions, depending on the hydrogen ion concentration of the solution.

The usual electrodialysis was carried out with the polarity of the cell arranged so that the lower membrane was the cathode, and with the pH of the solution about 3.7, the acid side of the isoelectric point. As has been stated, under these conditions, the sediment was always deposited at the lower or cathodic membrane. Under such experimental conditions, the cathodic deposition of the active material left two points open for discussion, namely, was the deposition on the cathodic membrane due to distinct cathode migration, or was it due to the material being drawn down to the lower membrane, which happened to be the cathode, merely by the force of gravitation.

In order to settle these points, experiments were performed with the initial pH of the solution at 3.7, but with the cell polarity reversed, thus making the lower membrane the anode, and the upper one the cathode.

TABLE 5

EXPERIMENT NUMBER	DESCRIPTION OF MATERIAL	MILLIGRAM PER KILO INJECTED	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	C BLOOD SUGAR AFTER 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D; (A − B) BLOOD SUGAR DROP ¾ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP 1¾ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
3	Filtrate	0.05	111	87	100	27	11	Produced by evaporation to constant weight at 30°C.
4	Filtrate	0.05	125	93	104	32	21	
15	Filtrate	0.05	127	82	111	45	16	
16	Filtrate	0.05	135	103	104	32	31	
12	Filtrate	0.05	139	129	132	10	7	Produced by adjustment of pH
16	Filtrate	0.05	126	103	122	23	4	
Average			127	99	112	28	15	

Under these conditions, the active material always migrated to the upper membrane against the force of gravity showing that when insulin solutions on the acid side of the isoelectric point were subjected to electrodialysis the cathodic deposition of active sediment was not due to gravitation, but was caused by a distinct cathodic migration of material carrying the activity.

The next point to be investigated was, obviously, the effect of changing the pH of the insulin solution from the acid to the alkaline side of the isoelectric point on the direction of migration of the active material. Experiments were performed with the pH of the insulin solutions adjusted to about 9.0 and kept on the alkaline side of the isoelectric point (pH = 4.8) by the continuous addition of dilute caustic soda solution. In all the experiments the deposition took place at the anodic, instead of at the cathodic membrane. Thus it has been shown that the deposition of an active sediment during electrodialysis of insulin solutions is caused by a definite migration of a material carrying the activity, and that the direction of migration is dependent upon the hydrogen ion concentration of the solution undergoing electrodialysis.

V. Deposition of the active sediment at a definite pH. In the preliminary experiments on the electrodialysis of insulin solutions, it had been observed that the pH of the solution changed as the electrodialysis progressed, as was to be expected, since the process combined dialysis with electrolysis. It was decided to study these pH changes more thoroughly with the idea of gaining perhaps more insight into the nature of the process which was causing the formation of the active sediment.

It has been shown in the previous section that the deposition of a sediment during electrodialysis of insulin solutions occurs on both the acid and alkaline side of the isoelectric point (pH = 4.8). The experimental difficulties involved in maintaining an alkaline solution during the electrodialysis, and more important, the rapid inactivation of insulin in alkaline solutions (Dudley, 1923; Shonle and Waldo, 1924) made it impossible to study the pH changes on the alkaline side of the isoelectric point. Therefore, only the pH changes on the acid side have been investigated.

The technique employed for the study of the pH changes during electrodialysis was of a type which not only followed every change in pH, but also determined the constancy of pH in different parts of the solution. The twofold result was accomplished by means of two capillary pipettes, one placed at the very bottom, and the other at the very top of the solution in the cell. By using a "Y" tube connection between the two capillary pipettes, it was possible to draw off liquid simultaneously from the very top and very bottom of the solution in the cell, thus minimizing the possibility of a change in pH by diffusion during the removal

of the samples. The pH of the top and bottom samples and also of the mixed solution was determined at different time intervals over a period of 24 hours by the colorimetric procedure previously referred to. The results obtained in three experiments on clinical U-20 insulin have been averaged, and are recorded in part A of table 6.

From these results it can be seen that when an insulin solution with an initial pH of 3.7 is subjected to electrodialysis there is a distinct drop of pH during the first part of the process, followed by a progressive increase in pH, and further, that these pH changes are the same in all parts of the cell within the experimental error of the pH determinations. It is

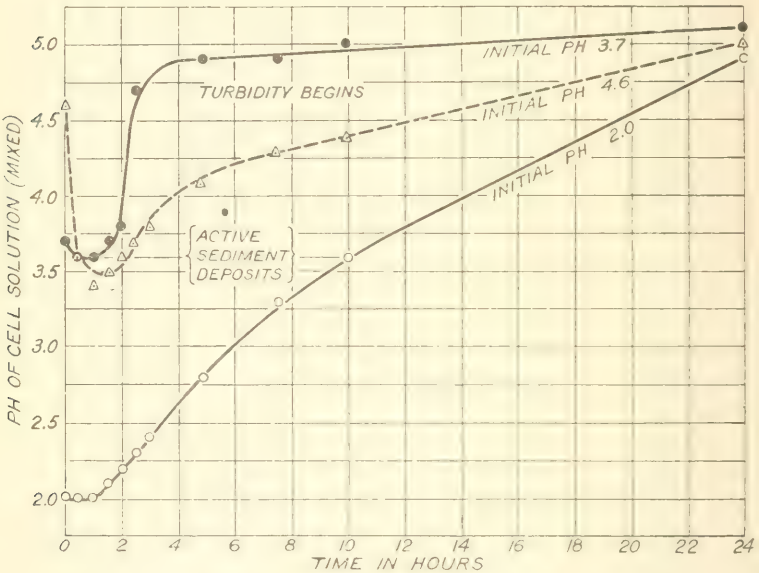

Fig. 5. pH changes during electrodialysis of insulin solutions

to be noted also, that accompanying the drop in pH of the solution is the deposition of all the weighable amount of active sediment. Furthermore, turbidity, followed by the formation of a boundary which very gradually settles down to the lower membrane, begins at a pH of about 4.7 to 4.8, or within the isoelectric range of the original insulin solution.

In order to determine whether these particular pH changes were specific for insulin solutions with an initial pH of 3.7, experiments were performed on solutions having a starting pH other than 3.7. Since the experiments recorded in part A of table 6 showed that the pH was constant throughout the entire cell, only the pH of the mixed solution was determined in the work which is about to be described.

TABLE 6

TIME	APPEARANCE OF SOLUTION	WEIGHT OF SEDIMENT DEPOSITED	[illegible]		
			At top of cell	On mixed solution	At bottom of cell
			Part A		
Start	Clear		3.7	3.7	3.7
½ hour	Clear	8.3 mgm.	3.6	3.6	3.6
1 hour	Clear	Not weighable	3.6	3.6	3.6
1½ hours	Clear		3.7	3.7	3.7
2 hours	Clear		3.9	3.8	3.8
2½ hours	Turbid		4.7	4.7	4.7
3 hours	Turbid				
5 hours	Turbid		4.9	4.9	4.9
7½ hours	Turbid		4.9	4.9	4.9
10 hours	Turbid		5.0	5.0	5.0
24 hours	Clear		5.1	5.1	5.1
			Part B		
Start	Clear			2.0	
½ hour	Clear			2.0	
1 hour	Clear			2.0	
1½ hours	Clear			2.1	
2 hours	Clear			2.2	
2½ hours	Clear			2.3	
3 hours	Clear			2.4	
5 hours	Clear			2.8	
7½ hours	Clear			3.3	
10 hours	Clear	7.3 mgm.		3.6	
24 hours	Turbid			4.9	
			Part C		
Start	Clear			4.6	
½ hour	Clear	7.2 mgm.		3.6	
1 hour	Clear	Trace		3.4	
1½ hours	Clear			3.5	
2 hours	Clear			3.6	
2½ hours	Clear			3.7	
3 hours	Clear			3.8	
5 hours	Clear			4.1	
7½ hours	Clear			4.3	
10 hours	Clear			4.4	
24 hours	Turbid			4.9–5.0	

Two experiments on clinical U-20 insulin with an initial pH of 2.0 and two on the same material but with an initial pH of 4.6, were performed, and the results obtained were averaged and recorded in table 6

parts B and C respectively. The data given in table 6 have been shown graphically in figure 5.

When the initial pH of the insulin solution was 2.0 nothing visible occurred during the course of the electrolysis until the pH had become adjusted to about 3.6, at which point practically the entire amount of active sediment deposited on the cathodic membrane, as was the case when the initial pH was 3.7. As the process continued, the pH of the solution gradually increased until, at a pH of about 4.8 the solution became turbid, as had been observed in the previous experiments.

When clinical U-20 insulin adjusted to an initial pH of 4.6 was electrodialysed, a very pronounced change in pH occurred during the first part of the process. The pH of the solution dropped from 4.6 to about 3.4 and all the weighable sediment was deposited on the cathodic membrane at a pH of about 3.6. As the electrodialysis continued, the pH of the solution progressively increased, until at a pH of about 4.8, the usual turbidity set in.

These results have led to the conclusion that the deposition of an active sediment during electrodialysis of insulin solutions having an initial pH on the acid side of the isoelectric point (4.8), always takes place at a definite pH, which appears to be about 3.6. It has also been concluded that the appearance in the solution of a turbidity at a pH of about 4.8, followed by the formation of a boundary which gradually settles to the lower membrane is due to an effect other than that which causes the deposition of the active sediment.

VI. Deposition of the active sediment is not due to isoelectric point precipitation. In the investigation of the pH changes in insulin solutions undergoing electrodialysis, it has been shown that the deposition of the active sediment takes place from a perfectly clear solution, when the pH has become adjusted to about 3.6. Furthermore, the clear solution becomes turbid at a pH of about 4.8. Since the isoelectric point of the original material is at a pH of about 4.8 (Doisy, Somogyi and Shaffer, (1923), (Council on Pharmacy and Chemistry 1923), and since the pH at which the deposition of the active sediment takes place is about 3.6, it appears, that the separation of the active material during electrodialysis of insulin solutions is not due to an isoelectric point precipitation.

The change in direction of migration of the sediment, as shown in section four, indicates that this material must be amphoteric. Until it can be demonstrated experimentally that the isoelectric point of the active sediment is at a pH other than 3.6, the argument advanced above is open to discussion because, if the isoelectric point is at 3.6 then the deposition of active sediment might be due to insolubility of the material at this particular pH. Several experiments were performed which prove that this deposition of active material was not due to isoelectric point precipitation.

In the first place, the isoelectric point of the active sediment was experimentally determined. Samples of dried sediment were dissolved in distilled water acidified with dilute hydrochloric acid. Very dilute alkali (N/200) was then gradually added with constant shaking until a precipitate appeared. The pH at which maximum precipitation occurred was determined colorimetrically and the addition of alkali was then continued until the precipitate dissolved, showing the amphoteric nature of the substance. Other samples of the same sediment were dissolved in distilled water, made alkaline with dilute alkali. The same procedure, as just described, was carried out excepting that dilute hydrochloric acid (N/100) was added instead of dilute alkali. The pH, at which the maximum precipitation occurred, or the isoelectric point of the active sediment, was found, as above described, to be about 5.2. Since the pH at which deposition of this material takes place has been shown experimentally to be about 3.6, while the isoelectric point of the sediment has been found to be at a pH of about 5.2, it can be seen that the deposition of active sediment during electrodialysis of insulin solutions is not due to an isoelectric point precipitation.

Other experimental proof for the validity of the above statement is had in the fact that if the circuit was broken just after the material had been deposited at a pH of about 3.6, the sediment entirely redissolved in a short time. This was to be expected, since this same active sediment is completely soluble in water at a pH of about 3.6.

Furthermore, if the insulin solution undergoing electrodialysis was agitated rapidly by stirring, no deposition of active sediment occurred even after two hours. When the stirring was stopped, the usual deposit formed. If an isoelectric point precipitation were the cause of the deposition of an active sediment during electrodialysis of insulin solutions, agitation of the solution should have no effect on the formation of the precipitate.

Therefore, it has been demonstrated by three independent experiments that the deposition of the active sediment from insulin solutions subjected to electrodialysis is not due to an isoelectric point precipitation.

The results of these experiments also show the difficulty of obtaining a filtrate with absolute physiological inactivity, due not only to the solubility of the active material at a pH of 3.6, but also to the overlapping of the isoelectric ranges (4.8 to 5.0 and 5.0 to 5.2) of the inactive and active constituents respectively.

The turbidity which has been shown to begin at a pH of about 4.8 and which is followed by the formation of a definite boundary and settling of the latter on the lower membrane, is probably due to isoelectric point precipitation of the inactive component. This pH (4.8 to 5.0) is the isoelectric point of insulin usually recorded in the literature (Doisy, Somogyi and Shaffer, 1923; Somogyi, Doisy and Shaffer, 1924; Dudley,

1923; Council on Pharmacy and Chemistry, 1923).] This was shown experimentally by the failure of the flocculated material to redissolve when the circuit was broken, and to almost immediate resolution upon the addition of either acid or alkali.

The relative inactivity of the material flocculated out of the solution of a pH of about 4.8, due probably to isoelectric point precipitation has been shown by a quantitative experiment. A solution of clinical U-20 insulin with an initial pH of 3.7, was divided into two equal volumes. Both portions were independently electrodialysed in the usual manner. The process was stopped and the sediment removed from one solution when the pH had become adjusted to about 3.6, and from the second at a pH of about 4.8. The solids from each were dried as usual and tested for physiological activity on the same weight basis. The results obtained are recorded in table 7.

From these results it can be seen that the material flocculated at a pH of about 4.8 showed an average blood sugar drop of only 47 and 42, as compared to 68 and 79 for the sediment deposited at pH 3.6. This experimentally indicated that the material which settles out of the solution at a pH of about 4.8, after the sediment has become deposited at a pH of about 3.6, contains little physiological activity. Contamination of the active sediment by this product materially decreases the potency of the active substance. This experimental evidence strongly indicates a fractionation of insulin by electrodialysis, as suggested in section three, and shows also that it is essential to stop the electrodialysis of insulin solutions before the pH has become adjusted to about 4.8, in order to prevent contamination and subsequent decrease in potency of the active material by the relatively inactive substance produced at this pH.

VII. Chemical investigation of the active sediment. In the previous sections it has been shown that an active sediment is formed during the electrodialysis of insulin solutions, from causes other than isoelectric precipitation. An investigation into the physical and chemical characteristics of the active substance has been undertaken with the hope of throwing some light on the chemical nature of the active principle.

The active deposit from insulin solutions subjected to electrodialysis is white when first prepared, but on exposure to the air, it gradually develops a grayish color. Under microscopic examination the active sediment shows sharp, ragged edges, like pieces of broken glass but no definite crystalline structure.

The active substance is soluble in dilute hydrochloric, sulfuric, and acetic acids, and in dilute caustic soda solutions. It is insoluble in absolute and 95 per cent ethyl alcohol, absolute ether, acetone, carbon tetrachloride, benzene, petroleum ether, and ethyl acetate. It shows slight solubility in absolute methyl alcohol saturated with dry hydrogen chloride.

TABLE 7

DESCRIPTION OF MATERIAL	MILLIGRAMS PER KILO INJECTED	A INITIAL BLOOD SUGAR IN MILLIGRAMS PER 100 CC. BLOOD	B BLOOD SUGAR AFTER $\frac{3}{4}$ HOUR IN MILLIGRAM PER 100 CC. BLOOD	C BLOOD SUGAR AFTER $1\frac{1}{2}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	D BLOOD SUGAR DROP AFTER $\frac{3}{4}$ HOUR IN MILLIGRAMS PER 100 CC. BLOOD	E; (A − C) BLOOD SUGAR DROP AFTER $1\frac{1}{2}$ HOURS IN MILLIGRAMS PER 100 CC. BLOOD	REMARKS
Sediment	0.05	128	80	82	48	46	Formed at pH 4.8
Sediment	0.05	116	77	84	39	32	
Sediment	0.05	119	66	70	53	49	
Average		121	74	79	47	42	
Sediment	0.05	158	102	108	56	50	Formed at pH 3.6
Sediment	0.05	138	46	18	92	120	
Sediment	0.05	154	88	72	66	82	
Sediment	0.05	119	60	56	59	63	
Average		142	74	63	68	79	

The sediment when dissolved in acidified water, imparts a pale yellow color to the solution. In alkaline solution this yellow color is much more pronounced.

The active material is precipitated from acidified aqueous solutions by phosphotungstic acid. It can also be precipitated from aqueous solutions by the addition of a large volume of absolute ethyl or methyl alcohol. If dry ether is added to a solution of the active substance in absolute methyl alcohol saturated with dry hydrogen chloride, a non-crystalline product is precipitated which shows slightly less than normal physiological activity as can be seen from experiment 5 recorded in table 4. The average sugar drop for the material precipitated as described was 40 and 54, as compared to 59 and 64, in the same time intervals for the control sample.

The material burns readily, giving rise to an odor resembling burning hair and leaves a white fluffy ash. Quantitative determinations for the percentage ash in several samples of sediment have been recorded in table 8. The ash determinations were made in a small platinum dish on samples ranging from 20 to 30 mgm. by ignition at red heat to constant weight. The ash is not alkaline, and its insolubility in dilute acids prompted a chemical investigation. This was done by the usual method (Scott, 1922; Treadwell and Hall, 1915) with sulfuric acid and hydrofluoric acid, used in determining silica. The results obtained have been recorded in table 8.

It can be seen from the tabulated data, that the ash from three different samples of sediment consisted entirely of silica, while the ash from the control, from which these sediments had been produced, consisted of only 60 per cent silica. Since the ash from the control samples showed a distinctly alkaline reaction toward phenolphthalein, which the ash from the sediments did not, it can be seen that the electrodialysis has removed an alkaline constituent which is not essential to the physiological activity of insulin preparations. Lack of material made it impossible to determine the ash content of the other samples of sediment recorded in table 8.

Attempts to remove the silica from the sediments were made by means of ultrafiltration through impermeable membranes and by re-electrodialysis with reversed polarity, thus drawing the sediment against gravity but these were not successful. The percent of silica in the samples before and after these treatments was practically the same. The relatively low ash in sediment no. 16, with no decrease in physiological activity, indicates that the silica is probably not a part of the molecule of the active principle in insulin preparations.

A test for nitrogen, sulfur, and phosphorus by means of sodium fusions on active sediments nos. 12, 13 and 16 was positive for the first two elements. No positive test for sulfur, phosphorus, or halogens could be obtained on the filtrate samples nos. 12 and 16 (pH 4.8 material).

TABLE 8

SAMPLE NUMBER	DESCRIPTION OF MATERIAL	PER CENT ASH NON-VOLATILE AFTER IGNITION	PER CENT ASH NON-VOLATILE AFTER $H_2SO_4 - H_2F_2$ TREATMENT	PER CENT SiO_2 IN ASH	PER CENT N_2 BASED ON TOTAL SOLIDS	PER CENT N_2 BASED ON ORGANIC SOLIDS	REMARKS
4	Control				14.15		Clinical U-20 iletin (Insulin-Lilly)
9	Control				13.89		
10	Control				14.21		
15	Control				13.51		
12, 13, 16	Control	3.82	1.53	60.00	12.35	12.84	Concentrated non-clinical iletin
4	Sediment				18.68		Made from clinical U-20 iletin (Insulin-Lilly)
9	Sediment				13.88		
10	Sediment				13.66		
15	Sediment				13.62		
12	Sediment	2.71	0.00	100.00	14.62	15.02	Made from concentrated non-clinical iletin
13	Sediment	2.50	0.00	100.00	14.12	14.48	
16	Sediment	1.30	0.00	100.00	13.87	14.06	
Average						14.52	
4	Filtrate				12.18		From U-20 clinical iletin
12	Filtrate				13.78		From concentrated non-clinical insulin
15	Filtrate				13.40		
16	Filtrate				12.11		

Each figure represents the average of 2 determinations.

The nitrogen content of samples of sediment and filtrate was quantitatively determined by the micro-Kjeldahl technique of Pregl (1923). A sample weighing between 11 and 12 mgm. was used for each analysis. The weighed sample was dissolved in that volume of N/10 sulfuric acid which would give a final concentration of 1 mgm. per cubic centimeter. Five cubic centimeters of this solution, measured by a calibrated pipette, were used for each determination. This procedure made it possible to get 2 analyses on each sample using 5 mgm. of material for each determination. The results obtained from several analyses on samples of control, sediment and filtrate have been given in table 8.

From these data it can be seen that the percentage of Kjeldahl nitrogen in the sediments analyzed based even on total solids, is fairly constant, while the nitrogen content for sediments nos. 12, 13, and 16 based on total organic solids is constant within 0.96 per cent, and averages 14.52 per cent for the three samples.

The amphoteric nature of the active sediment, as shown by its ability to migrate toward either the anode or cathode, depending on the pH of the solution, and also by its solubility in both dilute acids and alkalies, when coupled with its precipitation by phosphotungstic acid, and its average nitrogen content of 14.52 per cent makes it appear that the active principle of insulin either is a protein-like substance or is very closely linked to some protein. It is certain that the active material which deposits during the electrodialysis of insulin solutions, shows characteristic protein reactions, even though the active constituent in this sediment may not itself be a protein.

VIII. A micro-crystalline compound from the active sediment. The appearance of the active sediment formed from insulin solutions subjected to electrodialysis, when examined under a microscope, indicated possibilities for crystallization. The relative insolubility of the active material in all general organic solvents rendered the usual crystallization technique inapplicable. Advantage was taken finally of the solubility of the active sediment in acidified solutions.

Samples of active sediment were dissolved in dilute aqueous hydrochloric, acetic, and sulfuric acid solutions. These were permitted to evaporate slowly with the hope of obtaining the sediment in a crystalline condition. Several attempts were made but each time the material precipitated from the solution in an amorphous condition.

Another series of attempts was made using dilute sulfuric acid and ethyl alcohol as the solvent for the active sediment. Some of the solutions produced a micro-crystalline compound in very small quantity after standing for several days. The optimum condition for crystallization has not been established definitely, but equal volumes of 25 per cent sulfuric acid and 95 per cent ethyl alcohol produced crystals from two

batches of active sediment in about 24 hours. This was the shortest crystallization time ever obtained, the usual time being at least one week.

The formation of the crystalline compound from the active sediment occurs in a very peculiar manner. The sediment, when first dissolved, gives a clear, pale, yellow solution. The micro-crystals, in very small quantity, form in this acid solution after some of the alcohol has spontaneously evaporated. On longer standing, the crystals disappear, giving way to a turbid solution, from which an amorphous material precipitates. As more solvent evaporates this amorphous substance redissolves, giving a brownish colored solution from which, sometimes, on very long standing, the micro-crystalline substance is again formed. In several attempts, it has been impossible to get back the crystalline product even after standing for weeks. If a little ethyl alcohol is added to the liquid containing the amorphous substance after resolution of the crystals, the former re-dissolves, and in some cases, re-crystallization has been accomplished in this manner. However, if a little water is added to the liquid containing the amorphous substance, no resolution occurs, but even more amorphous material seems to come out of solution. If a large volume of water is added, complete resolution occurs but no crystals have ever been obtained from such diluted solutions.

The crystalline compound produced as described, has always had the same form, and has always shown the same relative insolubility in ordinary organic solvents and in water. The insolubility of the crystalline material in water, and the very small quantities in which it has been produced, together with the great difficulty experienced in attempting to free it from traces of mother liquor, have made the physiological and chemical investigation of the compound very difficult.

From the two microphotographs, figures 6 and 7, some idea may be had of the general appearance of the crystals under the microscope. Figure 6 shows a typical batch of crystals in contact with the mother liquor, while figure 7 shows a single group crystallizing in the usual radiating manner about some dust spot as a nucleus.

These crystals which are inactive are formed at the expense of the active sediment by the treatment described above. Their crystalline form suggests tyrosine. This aspect of the work opens up a line of attack on the insulin problem.

Discussion of errors. The greatest error in this investigation was involved in the physiological assay of the activity in the various samples of material. This biological error is not due to faulty technique or inaccurate methods, but is due to the different reactions of the experimental animals to the insulin injection. In order to establish the validity of the conclusions drawn from the physiological tests, a series of at least

three animals was run on each sample. The average error, calculated from the mean deviation for each series was found to be ± 16.19 per cent for the blood sugar drop ¾ hour after injection, and ± 18.98 per cent for the blood sugar drop 1¾ hours after injection. The error is larger for the second interval after injection because the results at this point are influenced to a greater degree by the individual reactions of the experimental animals. The error involved in weighing out the 2 mgm. samples for the physiological tests becomes negligible in view of the much larger animal error.

The lack of material necessitated the use of colorimetric procedure for the hydrogen ion determinations. Since in this investigation, the colori-

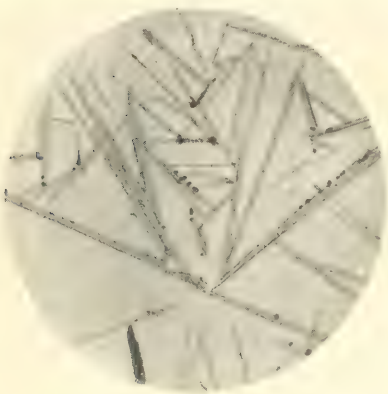

Fig. 6. Magnification 60×.
Ordinary light.

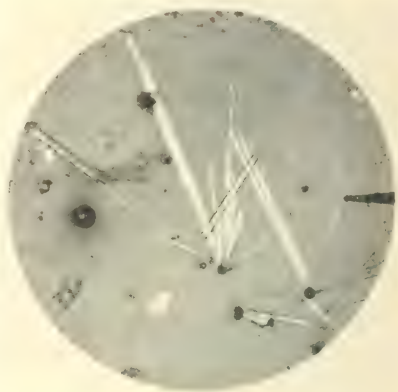

Fig. 7. Magnification 100×.
Green light.

Note: The authors wish to thank Mr. E. P. Polushkin of the Department of Metallurgy for these micrographs.

metric technique was open, not only to the usual optical and personal errors, but also to the so-called "protein" error, no greater accuracy than a close approximation is claimed for the hydrogen ion determinations. The results are, however, sufficiently accurate to indicate the general trend of the hydrogen ion changes in the cases investigated.

The largest error involved in the ash determinations is in the weighing of the samples (20 to 30 mgm.), and amounts to ± 0.33 to 0.50 per cent.

The micro-Kjeldahl technique was perfected by analyses on chemically pure samples of acetanilide. The accuracy of the method can be seen from the following typical analyses.

MATERIAL ANALYZED	WEIGHT OF SAMPLE	[illegible] OF ANALYSIS	[illegible] FORMULA	[illegible] ENCE FROM [illegible]
	mgm.			
	6.6	10.53	10.37	+0.16
	7.4	10.70	10.37	+0.33
c.p. acetanilid	7.4	10.82	10.37	+0.45
	7.2	10.82	10.37	+0.45
	7.1	10.70	10.37	+0.33

The standard sample weight in the actual analyses was 11 to 12 mgm. bringing into the method a possible error of ± 0.83 to 0.91 per cent.

All quantitative apparatus was calibrated and the errors involved were negligible in comparison to the errors discussed above.

SUMMARY

The results from A, ultrafiltration, and from B, electrodialysis have led to the conclusions summarized under the respective headings.

A. Ultrafiltration: 1. The active principle of insulin can be ultrafiltered through collodion membranes of high and low permeability without loss of activity.

2. The nitrogen content of insulin is not changed by ultrafiltration through collodion membranes of high or low permeability.

B. Electrodialysis: 1. An active sediment can be deposited from insulin solutions when subjected to electrodialysis.

2. The sediment produced by the electrodialysis of insulin solutions appears to be 100 per cent more active per unit of weight injected than the original material.

3. The filtrate, containing principally organic material, after removal of the active sediment, has little activity.

4. The electrode, at which the active sediment is deposited, is determined by the hydrogen ion concentration of the insulin solution undergoing electrodialysis.

5. There appears to be a definite hydrogen ion concentration at which the active sediment is produced from insulin solutions during electrodialysis.

6. The formation of the active sediment during the electrodialysis of insulin solutions, is not due to an isoelectric point precipitation.

7. The active sediment produced by the electrodialysis of insulin solutions shows protein reactions and contains organic sulphur but no phosphorus.

8. The inactive material shows protein reactions and contains neither sulphur nor phosphorus.

9. A micro-crystalline compound can be obtained from the active sediment formed by the electrodialysis of insulin solutions by treatment with an alcohol, sulfuric acid and water mixture.

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A CRITICAL EXAMINATION OF FOUR METHODS COMMONLY USED FOR THE DETERMINATION OF SUGAR IN BLOOD.

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The peculiar behavior of some of the well known blood sugar methods, when put to unusual uses, led us to the belief that it would be useful to add another to the already long series of critical studies of blood sugar methods.

It is a matter of common experience that, though a number of different methods may give concordant results with a given sample of blood, with a different blood the determinations may be quite at variance. This fact, that at times different methods agree while again they do not, is quite as interesting and probably more important than is the fact of their agreement, or lack of agreement, in itself. The methods which were examined were those of Benedict (1), Folin and Wu (4) as modified by Folin (5), Shaffer and Hartmann (11), and Hagedorn as described by Host and Hatlehol (8).

Because of the great number of possible causes for variation between the results obtained by the different methods, it was thought advisable to begin the study with a comparison of the behavior of the several methods towards a solution of pure glucose in water. In this way it would be possible to determine the precision and accuracy of each method with the fewest possible complicating factors, and any discrepancies would be more easily interpreted.

I. Results of the Work with Solutions of Pure Glucose.

A. Material and Apparatus.

The three following samples of glucose were used.

1. A sample prepared from white commercial glucose in 1915. This sample was purified by several precipitations from methyl alcohol. Until

used, it was kept in a desiccator over calcium chloride. Its rotating and reducing powers agreed with those of pure anhydrous glucose.

2. A sample prepared by crystallization from acetic acid (Hudson and Dale (9)). The Exose of the Corn Products Company was the starting point and the sample, when purified, was kept in a desiccator, as was Sample 1. This sample was prepared about 9 months before the first of it was used. Like the first sample its rotating and reducing powers corresponded with those of anhydrous glucose.

3. In a few instances Exose was used as purchased. When so used it is indicated in the tables (Table IV). Its rotating power indicated that it was 91.1 per cent anhydrous glucose. All solutions of this sample were so made that a 1 per cent solution would have the rotating power of a 1 per cent solution of anhydrous glucose.

The polarimetric determinations were made on a Schmidt and Haensch two-field instrument sensitive to 0.01° of angular scale. Solutions of about 5 per cent were used for polarimetric work. A mercury arc lamp with a filter giving a wave-length of 546×10^{-8} mm. was used as the source of light. As noted in the descriptions of Samples 1 and 2 the specific rotation of glucose for light of this wave-length was, granting the purity of our preparations, found to agree with that given by Quisumbing and Thomas (10) $[\alpha]^{25}_{546} = 62.02°$.

The anhydrous equivalent of the 5 per cent solution having been determined by rotation, a solution equivalent to 0.100 per cent anhydrous glucose was prepared from it by dilution. The reducing power of this diluted solution was then determined by the method of Quisumbing and Thomas (10). In all cases the agreement was within 0.3 per cent, whether the solutions were prepared from the purified samples or directly from Exose.

The colorimeter used was an ordinary Duboscq as made by Pellin. Readings were made by the use of the Chalet colorimeter lamp of Bausch and Lomb.

Inasmuch as we were primarily interested in the behavior of the several methods in blood sugar determinations, rather than in sugar methods in general, we have, in most instances, calculated our results to their respective blood sugar equivalents, instead of reporting the absolute indications of the sugar recovered. Thus when 0.20 mg. of sugar, absolute weight, is indicated by the Folin-Wu method it is reported as equivalent to a blood sugar of 100 mg. per 100 ml. The same absolute weight of sugar (0.20

mg.) by the method of Shaffer and Hartmann would indicate 40 mg. per 100 ml. of blood, by that of Benedict 31, and by that of Hagedorn 200. Or, again, in order to indicate 100 mg. per 100 ml. of blood there must be recovered by the method of:

	mg.
Folin and Wu	0.20
Shaffer and Hartmann	0.50
Benedict	0.64
Hagedorn	0.10

It will thus be seen that the requirements in sensitivity and precision of the different methods are quite varied. The difference in the significance of the absolute amounts of sugar recovered by the different methods depends upon differences in the amount of blood to which the volume of filtrate subjected to analysis corresponds. An aliquot part is used in all of the methods except that of Hagedorn. When it is desired to study the sensitivity of a method it becomes convenient to report the absolute weight of sugar indicated.

B. Benedict's Modification of the Lewis-Benedict Method.

With this method 2.0 ml. of blood, laked with 4.0 ml. of water, are treated with 19.0 ml. of a picric acid solution. The picric acid serves the double purpose of protein precipitant and oxidizing agent for the glucose. After the protein is precipitated, 8.0 ml. of the clear filtrate (equivalent to 0.64 ml. of the original blood) are treated with 1.0 ml. of 20 per cent sodium carbonate solution and placed in a boiling water bath for 10 minutes. At the same time, a tube containing 0.64 mg. of glucose in 4.0 ml. of solution, 4.0 ml. of the picric acid solution, and 1.0 ml. of the sodium carbonate solution is treated in like manner. After heating for the indicated time the tubes are cooled and the volume of each of the solutions made up to 12.5 ml. with distilled water. The blood sugar is then determined by comparing the amounts of picramic acid formed in the two tubes colorimetrically, it being assumed that under the conditions adopted this will vary directly as the sugar present. The reason for detailing the method in this way will become evident from what follows.

It is evidently assumed by the authors of the method that the

picric acid equivalent to that present in 6.5 ml. of the picrate-picric acid solution is removed with the protein precipitate, so that, after the precipitation is complete, the 8.0 ml. portion of the filtrate used for analysis contains the same amount of picric acid as does the 4.0 ml. added to the standard tube.

It is generally stated that this method gives fairly satisfactory results when used with normal bloods but that it is apt to give results which are too high, as compared with those obtained by other methods, when used with abnormal bloods, *e.g.* in diabetes. It may also give high results when used with bloods which may be presumed to have low protein content (Höst and Hatlehol (8)).

It may fairly be assumed that a reduced amount of protein in the blood will leave an excess of picric acid in the filtrate over and above that assumed by the author of the method to be present. Present knowledge does not permit us to write the equations for the reactions which occur in such a system but it would seem safe to assume that the principles of the mass law apply. Assuming that they do, it would follow that an increase, either of the oxidizing or reducing agent, the other in the meantime remaining constant, would lead to an increase in one or more of the end-products. If it be further assumed that in either case there would be an increase in the concentration of picramic acid, whether or not that of any or all of the other products increased, the observed discordant results would be at least partially accounted for. That is, bloods containing abnormally large amounts of sugar or an abnormally small amount of protein could be presumed to indicate a higher concentration of sugar with this method than would be the case with other methods, in which the balance between the oxidizing and reducing agents is not so easily disturbed.

To study this point experimentally determinations were made on sugar solutions containing (in 4.0 ml.) the glucose which theoretically would be present in the filtrates from 0.64 ml. of bloods containing respectively, 50, 75, 125, 175, and 200 mg. of sugar per 100 ml. of blood. The standard, with which each was compared, contained 0.64 mg. of glucose. The amount of the picrate-picric acid solution, in all cases, was the same as in the standard, 4.0 ml.

From Table I, it is evident that within a range of from 75 to 125 mg. per 100 ml., that is, within 25 per cent of the standard, there is a fairly close correspondence between the glucose actually

present and the glucose indicated by the picramic acid. The maximum error within this range is 1.3 per cent. As the concentration of glucose in the solution was further increased, however, more sugar was indicated than was actually present, there being a positive error of from 3 to 5 per cent. Likewise in the lower part of the range, when 50 mg. of sugar were present, only 47.9 were indicated, a loss of approximately 4 per cent. It seems from the nature of the experiment that the variations in the concentration of glucose, in the unknown from that of the standard, contribute to these discrepancies through the mass effect indicated

TABLE I.

Comparison of Solutions of Various Concentrations of Glucose with a Standard Solution Containing 0.64 Mg. The Effect of Concentration of Sugar upon the Amount of Picramic Acid Formed in the Lewis-Benedict Method of Determination of Sugar in the Blood.

No. of determinations.	Glucose to indicate blood sugar concentration in mg. per 100 ml.		Error: Deviation from glucose actually present.	
	Present.	Indicated.		
			mg.	*per cent*
	25.0	Colors could not be matched.		
6	50.0	47.9±0.5	−2.1	−4.2
4	75.0	74.0±0.8	−1.0	−1.3
4	100.0	100.1±0.2	+0.1	+0.1
4	125.0	125.2±0.5	+0.2	+0.2
8	150.0	156.1±0.7	+6.1	+4.1
4	175.0	181.5±1.4	+6.5	+3.7
8	200.0	210.0±0.9	+10.0	+5.0

in the above discussion. To study this point still further, solutions containing glucose equivalent to that in the filtrates from 0.64 ml. of bloods containing from 100 to 300 mg. per 100 ml. of blood were compared with a standard containing 1.24 mg. of glucose, *i.e.* double the ordinary strength and corresponding to a blood of 200 mg. per 100 ml.

From Table II it will be seen that, within a range of from 150 to 275 mg. per 100 ml., which is again roughly equivalent to a range of 25 per cent on either side of the standard of comparison, the greatest error is 1.9 per cent, and as a rule the error is much less than this. On the other hand only 90.7 mg. were recovered from

the 100 mg. solution, a loss of 9 per cent; while the 300 mg. solution indicated a concentration of 311.1 mg., a gain of nearly 4 per cent. The behavior of the 100 mg. solution when compared with the different standards is significant. It is of interest to point out at this time that with the advent of insulin and hypoglycemia the possible effect of low concentrations also becomes of importance.

In these two series of experiments, all solutions, containing a much greater concentration of glucose than did the standard of comparison, gave results which indicated a concentration greater than was known to be actually present, while all solutions con-

TABLE II.

Comparison of Solutions Containing Various Amounts of Glucose with a Standard Solution Containing 1.28 Mg. Which Is Equivalent to a Blood Sugar of 200 Mg. per 100 Ml. The Purpose is to Show the Effect of the Concentration of Glucose upon the Amount of Picramic Acid Formed.

No. of determinations.	Glucose present.	Glucose found.	Error: Deviation from glucose actually present.	
	Equivalent to blood sugar.			
	mg. per 100 ml.	*mg. per 100 ml.*	*mg.*	*per cent*
4	100.0	90.7 ± 1.3	−9.3	−9.3
4	150.0	147.2 ± 0.8	−2.8	−1.9
4	175.0	176.1 ± 0.2	+1.1	+0.6
4	200.0	199.8 ± 0.3	−0.2	−0.1
6	225.0	228.1 ± 1.1	+3.1	+1.4
4	250.0	250.4 ± 0.4	+0.4	+0.2
4	275.0	277.5 ± 1.1	+2.5	+0.9
5	300.0	311.1 ± 0.8	+11.1	+3.7

taining relatively much less glucose than the standard showed a corresponding loss in the amount indicated by the results of the determinations. Every case tested in this manner agreed with the results recorded in these two series. Hence it seems justifiable to conclude that the abnormally high results obtained by this method, as compared with results obtained by other methods, on bloods high in sugar content, may be due, in part at least, to this factor. That is, that the amount of picramic acid formed cannot be assumed to vary directly with the amount of glucose present, except over a very narrow range. This assumption is fundamental to all colorimetric methods of this type, and constitutes a weakness which must not be overlooked

Turning now to the effect of varying the concentration of the picric acid upon the amount of picramic acid formed, we carried out the following experiments. If the protein of the blood were decreased by 50 per cent the picric acid in the blood filtrate would be increased by an amount equivalent to that present in 3.0 ml. of the picrate-picric acid solution, and each 8.0 ml. portion of this filtrate taken for analysis would contain picric acid equivalent to

TABLE III.

Effect of Varying Amounts of Picric Acid upon the Amount of Picramic Acid Formed with a Fixed Amount of Glucose.

No. of determinations.	Glucose present 0.64 mg.	Glucose indicated.	Picrate-picric acid solution used.	Deviation from glucose actually present.
	Equivalent to blood sugar.			
	mg. per 100 ml.	*mg. per 100 ml.*	*ml.*	*mg.*
4	100.0	95.1 ± 0.5	3.0	−4.9
3	100.0	100.0 ± 0.5	4.0	±0.0
4	100.0	105.6 ± 1.0	5.0	+5.6
4	100.0	113.0 ± 1.2	5.5	+13.0

TABLE IV.

Comparison of the Behavior of Exose and Pure Glucose of Equal Concentration, as Determined by Rotation, Towards Benedict's Blood Sugar Reagent.

No. of determinations.	Glucose in solution. 0.64 mg. equivalent to 100 mg. per cent.	Glucose indicated.	Type of sugar used.
4	100.0	100.1 ± 0.2	Exose.
3	100.0	100.0 ± 0.5	Glucose 2.
4	200.0	209.1 ± 1.0	Exose.
4	200.0	213.3 ± 1.9	Glucose 2.

that in 5.0 ml. of the original solution rather than to that in 4.0 ml. as used in the standard tube, an increase of 25 per cent.

To determine the effect of such an increase in the amount of picric acid present, several samples, each containing 0.64 mg. of glucose in 4.0 ml. of water, were prepared. These samples were treated with varying amounts of the picrate-picric acid solution and after reduction were compared with a sample prepared in the standard manner. From Table III, in which the results of the experiment are recorded, it is apparent that any increase in the

amount of picric acid present in the unknown might well be responsible for an indication of extra sugar when compared with a normal standard.

In our hands the method has been reasonably precise; with the optimum concentrations the precision was about ± 1 per cent. Different observers in the laboratory were able to check each others readings within about the same range. Table IV shows the essentially similar reducing powers of "Exose" and of purified glucose when determined by this method.

TABLE V.

Behavior of the Folin-Wu Blood Sugar Method towards Various Concentrations of Glucose.

No. of determinations.	Glucose present.		Glucose indicated: Blood sugar equivalent.	Error: Deviation from glucose actually present.	
		Blood sugar equivalent.			
	mg.	*mg. per 100 ml.*		*mg.*	*per cent*
4	0.05	25.0	25.1 ± 1.2	+0.1	+0.4
4	0.15	75.0	74.9 ± 0.3	−0.1	−0.1
4	0.20	100.0	99.9 ± 0.7	−0.1	−0.1
4	0.25	125.0	125.9 ± 1.2	+0.9	+0.7
4	0.35	175.0	176.4 ± 2.1	+1.4	+0.8
5	0.45	225.0	228.7 ± 5.6	+3.7	+1.6
5	0.55	275.0	274.4 ± 2.8	−0.6	−0.2

C. Folin-Wu Method.

The Folin-Wu (4, 5) method in a sense stands midway between that of Benedict and that of Shaffer and Hartmann, since it is at the same time a colorimetric and a copper reduction method. We found it to give very accurate and precise results over an equivalent range of from 25 to 275 mg. per 100 ml. of blood, when used with solutions of pure glucose, the greatest error, 1.6 per cent, occurring at the upper end of the range. See Table V.

The manipulation we found to be easy, the solutions required are not difficult to prepare, and the colors obtained are of a quality and intensity which render them easy to compare. The method is also extremely sensitive. We were able to detect, and to measure, within 0.4 per cent, as little as 0.05 mg. of glucose, absolute weight. A solution of Exose which was equivalent to 0.100 per cent, as

determined by rotation, and which had a reduction value of 0.0997 ± 0.0011 per cent by the Quisumbing-Thomas method, was used as the unknown and was compared with a standard solution prepared as described by the authors of the method from Sample 2 described above.

D. Hagedorn Method.

The Hagedorn (Höst and Hatlehol 81) method is an iodometric method in which the oxidizing agent is potassium ferricyanide. The solution also contains potassium iodide. After reduction the solution is acidified and the unreduced excess of ferricyanide reacts with the hydriodic acid, formed on acidification, to liberate

TABLE VI.

Determination of Pure Glucose in Water Solutions by the Hagedorn Method.

No. of determinations.	Glucose present.		Glucose indicated: Blood sugar equivalent.	Error: Deviation of indication from glucose actually present.	
		Blood sugar equivalent.			
	mg.	*mg. per 100 ml.*	*mg. per 100 ml.*	*mg.*	*per cent*
5	0.025	25.0	25.3 ± 1.6	+0.3	+1.2
6	0.050	50.0	49.6 ± 2.3	−0.4	−0.8
6	0.075	75.0	74.4 ± 3.0	−0.6	−0.8
9	0.100	100.0	101.4 ± 1.8	+1.4	+1.4
6	0.125	125.0	124.8 ± 1.8	−0.2	−0.2
6	0.175	175.0	177.0 ± 2.1	+2.5	+1.4
11	0.225	225.0	237.7 ± 6.7	+12.7	+5.6
11	0.275	275.0	292.7 ± 4.8	+17.7	+6.4

free iodine. The amount of iodine so liberated is determined by titration with N/200 sodium thiosulfate. The method was found to be accurate over a range of from 25 to 200 mg. per 100 ml. Above the latter concentration, however, we were unable to confirm the authors' table. See Table VI.

The main advantages of this method over the ones more commonly used in this country appear to be that it requires but 0.10 of a ml. of blood and that it avoids the use of copper. This latter point would be of advantage only in special cases as for instance where copper is precipitated, as occasionally occurs with the filtrates of blood from the non-mammalians. However, the facts that only 0.01 ml. of the N/200 thiosulfate solution is equivalent

to about 1.8 mg. of glucose per 100 ml. of blood, and that the error in determining the end-point is of the same order of magnitude, make the precision of this method somewhat lower than that of the other methods studied. It possesses, on the other hand, extreme sensitivity, 0.025 mg. being readily indicated. For this reason it would have especial value where only small amounts of sugar are available for determination.

Because of the high dilution of the thiosulfate and the great significance of very small amounts of it, the sensitivity of the starch preparation used as the indicator becomes of considerable importance. It is also of importance that all of the solutions be freshly made, as was indeed recommended by the original author.

E. Shaffer-Hartmann Method.

This method makes use of the same blood filtrate as is used in that of Folin and Wu. 5 ml. of this filtrate (equivalent to 0.5 ml. of blood) are treated with an equal volume of the Shaffer-Hartmann reagent and placed in a boiling water bath for 15 minutes. At the end of this time the tubes are removed from the bath and cooled in running water. Each is then acidified and after a short time, allowed for the completion of the reaction, is titrated with N/200 thiosulfate solution. The value obtained, subtracted from that of a blank titration, gives the thiosulfate equivalent to the iodine which was used in reoxidizing the cuprous ion, and hence is a measure of the sugar which was originally present.

To study the behavior of this method, solutions of pure glucose were so made that 5 ml. of the respective solutions contained sugar corresponding to that of the filtrates from bloods in which the sugar ranged from 10 to 325 mg. per 100 ml. The results, expressed in ml. of N/200 thiosulfate, are given in Tables VII, VIII, and IX. In these tables the number of determinations which were made, the absolute quantities of glucose present, the concentrations of glucose in the blood to which these quantities correspond, as well as the thiosulfate equivalent as given in the tables of Shaffer and Hartmann are given. The tables show the method to be consistent with itself from day to day and with different solutions and different samples of glucose.

All of our determinations were made under conditions as nearly simulating those described by Shaffer and Hartmann as was pos-

sible, probably much more closely than would be the case in ordinary routine work. The titrations were made in a darkened room with the light from a Chalet colorimeter lamp reflected through the solution from below. The light for titration was thus con-

TABLE VII.

Determination of Pure Glucose in Water Solution by the Shaffer-Hartmann Method.

Glucose present.		N/200 thiosulfate equivalent to glucose present.						
			Actual titration.					
			Samples of glucose used.					
	Blood sugar equivalent.	Shaffer-Hartman table.	No. 2.	No. 1.	No. 1.	No. 2.	No. 2.	No. 2.
mg.	*mg. per 100 ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0.05	10.0	0.25	0.05	0.03		0.06		
0.10	20.0	0.50	0.07	0.07		0.11		
0.15	30.0	0.75	0.42	0.37		0.42		
0.20	40.0	1.00		0.70	0.76	0.74		
0.25	50.0	1.31	1.16	1.07	1.12	1.11		
0.50	100.0	3.15	3.08	2.97	3.01	3.03	3.04	3.03

TABLE VIII.

Determination of Pure Glucose in Water Solution by the Shaffer-Hartmann Method.

Glucose present.		N/200 thiosulfate equivalent to glucose present.				
			Actual titration.			
			Samples of glucose used.			
	Blood sugar equivalent.	Shaffer-Hartmann table.	No. 2.	No. 1.	No. 2.	No. 2.
mg.	*mg. per 100 ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0.375	75.0	2.25	2.07	2.00	2.02	
0.625	125.0	4.12	4.00	3.94	3.93	
0.875	175.0	5.96	5.91	5.92	5.86	
1.125	225.0	7.92	7.89	7.82	7.86	
1.375	275.0	9.79	9.81	9.81	9.86	9.89
1.625	325.0	11.83	11.93	11.94	11.92	

sistent from day to day. We feel that the end-point may be determined a little more precisely by reflecting the light through the solution in this manner than by the ordinary procedure. It should be emphasized, however, that the discrepancies between

our results and the table of Shaffer and Hartmann, which were revealed, in no way depended upon this method of lighting but have time and again shown themselves when the titration was done in ordinary daylight.

While the differences between our values and those given by the authors of the method are not large over the range of normal blood sugar, possibly not even significant, it will be noted that there is actual agreement only in the extreme upper part of the range. In the region of interest during insulin convulsions the difference

TABLE IX.

Compilation of All of Determinations of Pure Glucose in Water Solution by the Shaffer-Hartmann Method.

No. of determinations.	Glucose present.		N/200 thiosulfate required.		Mean deviation.	Deviation from Shaffer-Hartmann table.
		Blood sugar equivalent.	Shaffer-Hartmann table.	By titration.		
	mg.	*mg. per 100 ml.*	*ml.*	*ml.*	*ml.*	*per cent*
14	0.050	10.0	0.25	0.05	±0.03	80
14	0.100	20.0	0.50	0.08	±0.04	84
14	0.150	30.0	0.75	0.40	±0.04	47
11	0.200	40.0	1.00	0.73	±0.05	27
18	0.250	50.0	1.31	1.12	±0.05	14
12	0.375	75.0	2.25	2.03	±0.04	9.8
22	0.500	100.0	3.15	3.03	±0.04	3.6
11	0.625	125.0	4.12	3.95	±0.04	4.1
12	0.875	175.0	5.96	5.90	±0.04	1.0
12	1.125	225.0	7.92	7.86	±0.05	0.8
15	1.375	275.0	9.79	9.85	±0.06	0.6
12	1.625	325.0	11.83	11.93	±0.07	0.8

becomes significant and in the lower portions of the range the agreement between our results and those of Shaffer and Hartmann breaks down entirely.

Our attention was first directed to a possible inaccuracy in this range by Miss Hiller[1] of the Hospital of the Rockefeller Institute who had made simultaneous determinations with the Shaffer-Hartmann and the Hagedorn methods upon glycolyzing blood. The point made by Miss Hiller was that during glycolysis or fer-

[1] This work has since been published (7).

TABLE X.

Amounts of Glucose and Glucose Concentration of Blood Corresponding to Various Amounts of N/200 *Thiosulfate. Shaffer-Hartmann Method.*

Thiosulfate used. N/200	Actual glucose indicated.	Glucose in 100 ml. of blood.	Thiosulfate used. N/200	Actual glucose indicated.	Glucose in 100 ml. of blood.	Thiosulfate used N/200	Actual glucose indicated.	Glucose in 100 ml. of blood.	Thiosulfate used. N/200	Actual glucose indicated.	Glucose in 100 ml. of blood.
ml.	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*
0.10	0.100	20	3.10	0.509	102	6.10	0.902	180	9.10	1.282	256
0.20	0.120	24	3.20	0.523	105	6.20	0.914	183	9.20	1.294	259
0.30	0.135	27	3.30	0.537	107	6.30	0.926	185	9.30	1.306	261
0.40	0.150	30	3.40	0.551	110	6.40	0.938	188	9.40	1.318	263
0.50	0.165	33	3.50	0.565	113	6.50	0.950	190	9.50	1.330	266
0.60	0.180	36	3.60	0.578	116	6.60	0.963	193	9.60	1.343	269
0.70	0.195	39	3.70	0.591	118	6.70	0.976	195	9.70	1.356	271
0.80	0.210	42	3.80	0.604	121	6.80	0.989	197	9.80	1.369	273
0.90	0.220	44	3.90	0.617	123	6.90	1.002	200	9.90	1.382	276
1.00	0.235	47	4.00	0.630	126	7.00	1.015	203	10.00	1.395	279
1.10	0.245	49	4.10	0.643	129	7.10	1.028	205	10.10	1.407	281
1.20	0.260	52	4.20	0.656	131	7.20	1.041	208	10.20	1.419	283
1.30	0.275	55	4.30	0.669	134	7.30	1.054	211	10.30	1.431	286
1.40	0.290	58	4.40	0.682	136	7.40	1.067	213	10.40	1.443	289
1.50	0.300	60	4.50	0.695	139	7.50	1.080	216	10.50	1.455	291
1.60	0.315	63	4.60	0.708	141	7.60	1.093	219	10.60	1.467	293
1.70	0.330	66	4.70	0.721	144	7.70	1.106	221	10.70	1.479	296
1.80	0.345	69	4.80	0.734	147	7.80	1.119	223	10.80	1.491	298
1.90	0.355	71	4.90	0.747	149	7.90	1.132	226	10.90	1.503	301
2.00	0.370	74	5.00	0.760	152	8.00	1.145	229	11.00	1.515	303
2.10	0.383	77	5.10	0.773	155	8.10	1.157	231	11.10	1.527	305
2.20	0.396	79	5.20	0.786	157	8.20	1.170	234	11.20	1.539	307
2.30	0.409	82	5.30	0.799	159	8.30	1.182	236	11.30	1.551	310
2.40	0.422	84	5.40	0.812	162	8.40	1.193	239	11.40	1.563	313
2.50	0.435	87	5.50	0.825	165	8.50	1.205	241	11.50	1.575	315
2.60	0.447	89	5.60	0.838	167	8.60	1.218	243	11.60	1.587	317
2.70	0.459	92	5.70	0.851	170	8.70	1.231	246	11.70	1.599	319
2.80	0.471	94	5.80	0.864	173	8.80	1.244	249	11.80	1.611	322
2.90	0.483	97	5.90	0.877	175	8.90	1.257	251	11.90	1.623	325
3.00	0.495	99	6.00	0.890	178	9.00	1.270	254	12.00	1.635	327

mentation experiments the Hagedorn method still indicated a concentration of about 20 mg. per 100 ml. when the Shaffer-Hartmann method first registered zero. This was soon and abundantly confirmed in our own laboratory. It was in fact this circumstance which led to the present study. In addition to studies in glycolysis the lower portions of the table are of importance in glycogen determinations, in the study of blood sugar in the invertebrates, and doubtless in other phases of carbohydrate metabolism.

Table X is constructed from the results recorded in Tables VII and VIII by interpolation and is offered as a substitute for Table II, p. 380, of Shaffer and Hartmann. It is very strongly urged, however, that each investigator who uses this method construct his own table, rather than use either ours or the one of the original authors.

II. Results of the Work with Blood.

A few experiments have been carried out with blood in order to determine to what extent the results described in Part I of this paper may explain the variations which have been noted in practical blood sugar analyses.

Oxalated beef blood was used in these experiments. As soon as the blood was received from the slaughter-house it was strained through coarse gauze to remove any small clots which might have formed and then it was kept for use at 2°C., except while samples were being removed for analysis. The sugar content was first determined simultaneously by each of the four methods. The results are given in Table XI. From this table it will be seen that the first three methods gave almost identical results but that the Shaffer-Hartmann method, using their own table, indicated but 117 mg. of glucose per 100 ml. of blood as against 120 or more as determined by the other methods. If our table is used the value is brought up to 120 and compares favorably with that indicated by the other methods.

In order to determine the behavior of the Shaffer-Hartmann method in the lower ranges, simultaneous determinations were made on 5 ml. of the blood filtrate and on 2 ml. which had been diluted to 5 by the addition of 3 ml. of water. From 2 ml. 40 per cent of the glucose indicated in the 5 ml. samples should be

recovered. Two trials were made, with the results shown in Table XII. It is evident from these experiments, quite apart from our other work and quite in accord with the work of Hiller, Linder, and Van Slyke, that the table of Shaffer and Hartmann

TABLE XI.

Sugar Recovered from a Single Sample of Blood by the Four Different Methods.

Method.	No. of determinations.	Sugar content of blood.
		mg. per 100 ml.
Benedict	4	121.4 ± 1.2
Folin-Wu	4	122.9 ± 1.6
Hagedorn	5	120.2 ± 2.3
Shaffer-Hartmann. (Authors' table.)	4	117.1 ± 1.2
" Our "	4	120.1 ± 1.2

TABLE XII.

Comparison of the Behavior of the Shaffer-Hartmann Method when Used with Two Different Amounts of Glucose in a Blood Filtrate.

	No. of determinations.	N/200 thiosulfate.	Mg. glucose per 100 ml. of blood, calculated from:	
			Shaffer-Hartmann table.	Table X.
		ml.		
Sample A.				
5 ml. of blood filtrate	4	3.54	110.1 ± 1.3	113.9 ± 1.3
40 per cent of above			44.0 ± 1.3	45.6 ± 1.3
2 ml. of blood filtrate	3	0.86	34.5 ± 1.2	43.5 ± 1.2
Difference			9.5 = −21%	2.1 = −4.6%
Sample B.				
5 ml. of blood filtrate	4	3.27	103.0 ± 1.2	106.5 ± 1.2
40 per cent of above			41.2 ± 1.2	42.6 ± 1.2
2 ml. of blood filtrate	4	0.88	35.0 ± 2.1	43.7 ± 2.1
Difference			6.2 = −15%	1.1 = +2.6%

is not consistent over the range here used. It is apparent, also, that at the blood sugar level of insulin convulsions, which was approximated in the 2 ml. samples, the error arising from the use of the original tables is considerable, of the order of 15 or 20 per cent of the total sugar present.

In Part I it was shown that the amount of glucose which is indicated by Benedict's method depends upon the relative amounts of glucose in the standard and in the unknown solution and upon the amount of picric acid present (Table III). It might be supposed that any large decrease in the amount of protein present would increase the amount of picric acid left to oxidize the glucose.

To test this the sugar in a fresh sample of beef blood was determined by the Benedict method (as described by the author) and found to be 107 mg. per 100 ml. of blood. A solution of pure glucose of this concentration was prepared in 0.9 per cent sodium chloride solution. 70 ml. of the original blood were diluted to 100 ml. with this solution. In this manner the sugar concentration was left unchanged while that of the protein was reduced 30 per cent. The amount of picric acid in the filtrate should then be increased by 30 per cent of the amount usually removed in precipitating the protein; that is, 30 per cent of the picric acid which would be in 7.0 ml. of the picrate-picric acid solution. This would increase the picric acid in an 8.0 ml. portion of the filtrate by an amount equal to that in 0.63 ml. of the picrate-picric acid solution, an increase of 16 per cent in the available picric acid. We have shown, Table III, that an increase of 25 per cent in the available picric acid causes the indication of 5.6 per cent of extra glucose, so that the increase here would be 3.6 per cent of 107 or 3.8 mg. of extra glucose per 100 ml. of blood.

$$\text{Calculation: } \frac{16}{25} \times 0.056 \times 100 = 3.6.$$

We would expect, then, to find indications for about 110 mg. of sugar per 100 ml. of the blood which had been diluted. The following shows the results of such an experiment.

Original blood unmodified, Benedict method	106.7 ± 2.8
Protein decreased 30 per cent. Theoretical increase in sugar indicated	3.8
Theoretical indication for diluted blood	110.5
Sugar content of diluted blood as actually found	111.0 ± 1.6

From the figures just given it is evident that our supposition is at least fair, and that any considerable decrease in the blood proteins may be expected to increase the apparent sugar content of the blood when the latter is determined by this method.

To 100 ml. of this same original blood 100 mg. of pure glucose were added, making a total glucose content of the blood of 0.2067 per cent. From Table IV we would expect 14 mg. of extra glucose to be indicated, so that by this method there should be an indicated concentration of 206.7 + 14.0 = 220.7 mg. per 100 ml. of the modified blood. The experimental figures are tabulated below.

Original blood, unmodified Benedict method	106.7 ± 2.8
Modified blood, 100 mg. glucose added to the above	206.7
Increase expected from results in Part I	14.0
Expected indication	220.7
Actual indication	221.6 ± 1.4

It is thus evident that the results with the blood agree favorably with those obtained from the solutions of pure glucose in Part I of the present paper. In further support of this conception are certain results obtained by Höst and Hatlehol (8) who compared the Bang, Hagedorn, Folin-Wu, and the Myers and Baily modification of the Lewis-Benedict methods. They determined the glucose of normal human bloods and found that the four methods agreed fairly closely. When glucose was added, however, so that the glucose content was actually in the neighborhood of 0.200 per cent, the Benedict method usually indicated an excess of from 6 to 14 mg. over the other methods, which agrees well with our results.

DISCUSSION.

We have not attempted to define the nature of the reducing substances in the blood but have considered the behavior of the several methods, first towards a solution of pure glucose and then towards the effective reducing substances of the blood considered as pure glucose.

It has been shown that errors may arise during the progress of blood sugar determinations which are inherent in the method used. These errors may be due to the relative amounts of the reacting substances present in the system, or to other causes. It has been shown that some of the discrepancies between different blood sugar methods need not necessarily be ascribed, as is so often done, to hypothetical reducing substances other than glucose, which are presumed to be in the blood. Thus the extremely high sugar content sometimes indicated by Benedict's method for diabetic

blood need not necessarily be ascribed to creatinine but may simply be the direct result of the high glucose concentration.

An increase in the oxidizing agent may likewise increase the amount of sugar indicated. It is possible that both of these effects and especially the latter might be reduced if the final concentration of the oxidizing agent could be increased. In line with this suggestion it is interesting to note that Calvert (2, 3) found the Folin-Wu method to behave in a somewhat similar manner when the alkaline-copper-tartrate solution was diluted, that is, when the concentration of the oxidizing agent was reduced. Stanford and Wheatley (12) point out that a large excess of the copper tartrate is necessary if the reduction is to be strictly parallel to the amount of sugar present. Apparently one of the factors in the success of the Folin-Wu method lies in the great solubility of the oxidizing agent chosen.

Shaffer and Hartmann's method has been found to be very satisfactory to work with when its limitations are known and recognized. The technique is simple and the results are consistent. A lack of sensitivity to very low concentrations of glucose however, distinctly limits its field of usefulness. The origin of this loss of sensitivity apparently arises from the introduction of the iodine salts or of the oxalate, as otherwise their solution is very similar to that of Folin and Wu, which is apparently much more sensitive.

Haskins and Holbrook (6) have recently offered a modification of this method for clinical use. Altogether their modifications seem to have decreased the reliability of the method without contributing much towards its convenience.

SUMMARY.

1. The behaviors of four different methods for the determination of blood sugar have been compared.

2. The Folin-Wu and Hagedorn methods were found to be accurate and fairly precise when used to determine the concentrations of pure glucose solutions approximating those encountered in blood analysis.

3. It was found that an increase in the concentration of either the sugar or of the picric acid in the Benedict method would lead to high results, and this is offered as at least a partial explanation

of the high results obtained by this method in certain pathological bloods.

4. The Shaffer-Hartmann method was found to be reliable and satisfactory for concentrations above about 25 mg. per 100 cc. of blood. Certain corrections also appear to be necessary in their table. A substitute table is submitted.

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SOME OF THE POTENTIAL PUBLIC HEALTH HAZARDS FROM THE USE OF ETHYL GASOLINE*

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THE Laboratory of Industrial Hygiene of Columbia University was requested by the Medical Committee of the Ethyl Gasoline Corporation to undertake an investigation as to the possible hazards that might arise from the use of tetraethyl lead in the gasoline sold to automobilists.

Tetraethyl lead is a colorless liquid with a specific gravity of 1.62 at 15°C., boiling at 200°C., but not without some decomposition. It is a lipoid solvent, attacks rubber, and is insoluble in water. The fact that it is a lipoid solvent brings us face to face with a possible lead hazard through skin absorption. When exposed to sunlight or evaporation, it decomposes and forms as one of its decomposition products triethyl lead, a poisonous compound whose pharmacologic properties have been studied by Harnack.[1] When used in the automobile, it is added to gasoline, together with an organic halide, in the proportion of 1,300 parts of gasoline to 1 part of the lead compound; this mixture being known to the trade as ethyl gasoline. The purpose of adding the organic halide is to assist in the removal of the lead from the motor after combustion. When the Laboratory at Columbia University was requested to undertake this study, it was still proposed to use the 1,000 to 1 mixture.

As the United States Bureau of Mines had already been studying for some months the possible hazard to the public from the lead in the exhaust gases of engines using this ethyl gasoline, the Medical Committee sug-

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[1] Harnack, E.: Ueber die Wirkungen des Bleis auf den thierischen Organismus. Arch. f. exper. Path. u. Pharmakol., 1878, *9*, 158.

gested that Columbia University confine their efforts to the following hazards:

1. The possible hazard to the tank man, garage man, or anyone who might get a few drops of the concentrated material on his clothes or person from time to time when handling tetraethyl lead.

2. The possible hazard that might arise from splashing the ethyl gasoline (1,000 to 1 mixture) on the person; or from the cleansing of the hands or clothes by the mechanic or by the housewife; or while adjusting the carburetor or cleaning out the gas line or tank; etc.

3. The possible danger from the inhalation of ethyl gasoline fumes from spillage in the garage and other places where vaporization may take place.

Before our experiments were productive of results, an unfortunate experience occurred in an experimental plant of the Standard Oil Company of New Jersey at Bayway, which was engaged in a study of an improvement in the method of manufacture of tetraethyl lead. A number of the employees working in this plant became poisoned at one time and were placed under observation at the Reconstruction Hospital, New York City. Before their removal to the Hospital, one of the men died. The symptoms of the worst cases were those described by several writers in cases of acute lead poisoning and were very similar to those produced by ethyl alcohol. Indications of profound cerebral disturbances were present in the more severe cases. The victim suffered from persistent insomnia and became delusional, extraordinarily restless and talkative. There was an exaggerated movement of all muscles of the body. The patient became maniacal and died of exhaustion. Four of the men transferred to the Hospital died within a few days. The rest of the men were discharged from the Hospital after receiving the proper treatment.

We took advantage of the opportunity to study these cases at the Reconstruction Hospital so that we would be in a better position to interpret our animal work. We found lead in the stools of eighteen out of twenty-nine men that were examined. This continued to be present for several months after removal from exposure. We could not detect any lead in the sweat of men subjected to thermal baths. Tests were also made on the skin with ammonium sulphide, but the findings were negative. Several specimens of spinal fluid also gave negative results. Stippled cells were found in the blood of nine out of twelve men examined by this Laboratory. The findings of the laboratory at the Hospital on a larger group of men indicated that 50 per cent. of the men who had been exposed showed stippled cells in the blood.

The report of the chief examiner of New York City on the autopsies performed on the four men who died at the Hospital reveals no gross pathologic findings. No one would have been able to say that lead was the cause of their deaths without a chemical analysis or without knowledge of where the men had worked. This of course is in agreement with our past knowledge as to the pathologic and histologic findings in lead cases.

The autopsies determined the cause of death "pending chemical examina-

tion and investigation as 'hemorrhagic broncho-pneumonia, visceral congestion' in two cases; 'hemorrhagic broncho-pneumonia, secondary anemia' in the third; and in the fourth 'general visceral congestion.'" We have given these data as an aid to a better understanding of our own findings.

After the unpleasant accident at Bayway the Ethyl Gasoline Corporation decided to remove any possible hazard to the general public, including the garage man, from any contact with the concentrated tetraethyl lead, by delivering only the 1,300 to 1 mixture to the filling stations. As a further precaution, it is intended to add a red dye to the gasoline so as to lessen the possible hazard through skin absorption when handling the dilute mixture. This dye would act as a deterrent to the housewife or the mechanic who might otherwise be tempted to use some of the gasoline for cleansing purposes.

This precaution would also serve as a warning against the use of ethyl gasoline in gasoline stoves for cooking purposes, as well as in the gasoline torches used by painters, plumbers, etc. We have seen evidence that fine lead is given off when this gasoline is burned in such apparatus and that this fine lead remains in suspension for several days. Just what the extent of this particular hazard is, we cannot say as we have made only the most preliminary tests. But it will be necessary to educate the public—if it is possible—to use ethyl gasoline only in the automobile.

Procedure in Animal Experiments

Chemicals

The tetraethyl lead used in these experiments was furnished by the Medical Committee of the Ethyl Gasoline Corporation and was supposedly the pure product. We purchased our gasoline from a filling station in the neighborhood of the Medical School and made up our own mixture from time to time as we needed it.

Animals

We used matured rabbits, guinea-pigs, rats, goats, pigeons, and monkeys in our work. The animals were kept under observation for a period of time before they were subjected to any exposure, and their weights and temperatures were noted during the whole series of experiments. The red and white and differential cell counts were made periodically on the blood of the goats and the rabbits. The blood smears were carefully examined for stippled cells. The control animals were kept away from the exposed animals so that they would not have a chance to accumulate lead in any way. Outside of this precautionary measure, all of the animals were treated in the same manner. Samples of feces were collected from time to time and examined for lead.

Method of Examination

Blood.—Three stains were used in examining the blood for stippled cells—Wright's, Glibert's, and Harlow's methods, as used and recommended by the Division of Industrial Hygiene, Provincial Board of Health of Ontario.

Lead.—The bodies were burned at a low temperature over a gas flame so as to avoid any volatilization of the lead. The chromate method de-

scribed by Fairhall was used in the actual determination of the lead. We found it necessary to make our solution almost alkaline if not alkaline, in order to be sure that the lead was precipitated out by the hydrogen sulphide. This, of course, necessitated the redissolving of the sulphide precipitate in a small amount of nitric acid and a reprecipitation by hydrogen sulphide.

Skin Absorption Tests

Dosing of the Animals

We determined at the beginning of our work that a man would have approximately 10 c.c. of gasoline left on his hands after washing them and before drying them on waste or a towel. Using this as a basis, we dosed our animals in the same proportion by weight, taking the weight for man as 70 kilos. We felt that this was as near as we could come to duplicating an industrial condition. Of course a man in a garage may wipe his hands dry, but our observations have led us to doubt it unless he is washing up preparatory to going home. Generally the last traces dry off by vaporization. In the case of a spillage the exposure would be much greater, and the average man will not change his garments if gasoline spills on them, preferring rather to let the gasoline evaporate. However, one may be sure of a greater absorption with the larger exposure if lead storage took place with our comparatively small daily exposure. Even if the man's exposure should not occur daily, there is bound to be some storage of lead as the rate of excretion does not equal the rate of absorption. Whether the exposures will be frequent enough or great enough to make this storage of lead a factor in the man's health is a matter for the health officer and the doctor to determine as they come in contact with those exposed.

We applied the ethyl gasoline to the skin by means of a small pipette. The gasoline was permitted to flow slowly from the pipette on to the uninjured skin by pushing the hair aside whenever it was possible. When we found it necessary to shave the animal, several days were allowed to elapse to permit the skin to heal before it came in contact with any gasoline. We took care in all of our work never to apply the ethyl gasoline to any broken skin. Also, because our observations showed that the gasoline affected the cutaneous tissue, we varied the surface area of the gasoline application from day to day. We did not wipe off the gasoline from the skin once it had been applied, but permitted it either to vaporize or to be absorbed. We felt that our problem consisted in determining whether lead could be absorbed from the ethyl gasoline into the body. If lead is shown to be absorbed, the precaution of wiping off any gasoline that might come in contact with the skin is a part of the educational program that must be undertaken if ethyl gasoline becomes very generally used. We exposed the animals daily except on Sundays and holidays and when feces were being collected.

We were very skeptical at the beginning of our work as to whether or not there was any skin absorption, but felt rather that the actual poisoning took place through ingestion or inhalation. Rabbits and guinea-pigs have the habit of cleaning their fur

with the tongue, and they can be seen doing this not only to their own bodies, but to other animals of the same species. We tried to overcome this by placing jackets on the animals, but these jackets got torn and observations led one to believe that they interfered with the physiologic activities of the animal—probably the heat regulating mechanism. At any rate, the normal animals died as readily as the exposed ones. On account of this fur licking habit, we decided to use goats as well as the smaller animals in our work. The goat is a hardy animal, not susceptible to pulmonary diseases, and, as far as we have observed, has no tendency to lick itself.

Test Experiment

In order to determine whether absorption actually did take place through the skin and not by ingestion or inhalation, we decided to make the following test, which was repeated at three different times.

A cat was anesthetized with ether. Tracheotomy was performed and a cannula placed in the carotid so that the blood pressure and the respiration could be observed. The tube from the trachea was led away from the body in such a way as to preclude any chance for the animal to inhale any fumes. In order further to safeguard against the breathing in of any tetraethyl lead fumes, an exhaust fan was placed near the abdomen and an electric fan at the head of the cat. Six cubic centimeters of the concentrated tetraethyl lead were allowed to flow from a pipette on to the clipped abdomen and a record was taken on the kymograph. As far as our records show, there did not seem to be any change in the blood pressure or the respiration. The cats died at different intervals, varying from eleven minutes to two hours. A slight abrasion was noticed in the skin of the animal dying in eleven minutes. As soon as the cat died the skin was cleaned with alcohol, to remove any tetraethyl lead remaining on it. The skin was quickly removed and the abdomen opened for examination. In each case blisters were observed all through the abdominal region and the intestines had a peculiar bluish hue. The odor of the tetraethyl lead was quite distinct when the body was first opened. After this gross examination, the whole body with the exception of the skin was burned and the lead contents determined. The average lead content of the three cats used in this experiment was found to be 0.651 gm. of lead. Probably the lead contents would have been greater if it had not been for the excessive ventilation maintained to prevent any possible inhalation of the fumes.

This experiment would seem to indicate that there is no doubt that this lead compound is readily absorbed through the skin and that it must be immediately washed off the skin with green soap and kerosene if it should by any chance get on the body. Water and ordinary soap will not remove it. The Ethyl Gasoline Corporation is to be commended on its action in removing this concentrated compound from the public.

Summary of Hazards from Skin Contact

Rabbits, guinea-pigs, and goats were used in this part of the study which had to do with the possible hazards that may exist from ethyl gasoline

coming in contact with the skin and a subsequent absorption into the body of the minute quantity of the lead compound present in the gasoline.

We are giving a summary of our findings because, as we have stated before, the problem consists in determining whether lead can be absorbed and stored in the body. It is not necessary for us to prove that the animal died of lead poisoning or how much lead the organism can store before any marked pathologic symptoms appear. The individual idosyncrasies vary too much to make these facts of supreme importance. Furthermore, there is no doubt that there are many persons engaged in the industries who are subject to lead exposures. The storage ability and excretory power of these persons may just balance the lead intake and any excess exposure may be enough to push them over to the wrong side of the line.

Experiments on Rabbits:

Each animal received 0.3 c.c. of the 1,000 parts of gasoline to 1 part of tetraethyl lead mixture practically daily until it died. The results of these experiments are given in Table 1. The number of exposures that the animal underwent varied from 6 to 187. Twenty-eight animals have died. The average findings on these animals are:

	Temperature	*Weight*	*Red Blood Count*
	°C.	*kilos*	*per c.mm.*
Initial.....	38.9	2.07	4.88
Final.....	38.8	2.03	4.86

Stippled cells were found in the blood of fourteen of the rabbits. These rabbits did not show any stippled cells at the beginning of their exposure. The two rabbits which died after they had been treated but 6 and 13 times, respectively, showed only traces of lead in their carcasses after they had been burned. An average of 1.93 mg. of lead per kilo of body weight was found to be present in the carcasses of the rabbits that died in this part of the study. The quantity of lead accumulating in the body does not always follow the number of exposures to which the animals are subjected; but there seems to be no doubt as to the tendency for an increased amount of lead storage in the body with the longer exposures.

Excretion.—The average rate of excretion as shown by frequent examination of the feces is approximately 0.044 mg. per twenty-four hours, and, so far as our observations go, does not seem to increase with a greater storage.

Behavior.—The animals did not show any signs of ill health during the exposure until several days before they died. During the last two or three days before death the animal as a general thing became quiet and frequently hunched up as if suffering from colic. If the animal was removed from the exposure as soon as the first symptoms of ill health appeared, it soon recovered. Four of the animals developed tremors and spasms just before the end. One of the rabbits which had received 185 applications became completely paralyzed in the hind quarters two days before death. This rabbit showed 4.9 mg. of lead per kilo of body weight. Several of the rabbits aborted, but the fetuses were not examined for lead.

Autopsy.—An autopsy was performed on each animal that died and revealed nothing more than was found

in the control animals when they were killed or died except that there was the consistent presence of a white blood clot in the right auricle of the leaded animals. This was also observed in several supposedly sound animals. The ante mortem clot is usually accounted for by a slowing down of the circulation. If this is the correct explanation it will corroborate what has been observed on men who have been exposed to the compound, namely, a lowered blood pressure. One man, in whose twenty-four-hour urine sample we had found 12 mg. of lead, had a systolic pressure of 90.

TABLE 1.—RESULTS OF EXPERIMENTS ON TWENTY-EIGHT RABBITS THAT DIED AFTER BEING TREATED WITH ETHYL GASOLINE

(Dosage: 0.3 c.c. of 1,000 parts gasoline to 1 part tetraethyl lead)

NUMBER OF ANIMAL	NUMBER OF DOSES	TEMPERATURE		WEIGHT		RED BLOOD COUNT		STIPPLING[1]	LEAD PER KILO OF BODY WEIGHT
		Initial	Final	Initial	Final	Initial	Final		
		°C.	°C.	*kilos*	*kilos*	*per c. mm.*	*per c. mm.*		*mg.*
8[2]	85	39.4	39.2	1.53	1.70	4.58	4.82	+	3.44
9	49	39.2	38.5	3.31	2.12	5.20	5.20	+	0.78
12[2]	44	39.0	38.9	2.17	1.76	5.72	5.31	−	0.86
13	85	39.0	38.9	1.63	1.71	4.09	4.64	+	3.23
14[3]	185	39.0	39.2	2.35	3.13	4.35	4.72	+	4.90
16	52	39.0	38.8	1.57	1.87	4.68	4.61	−	0.85
17	77	39.0	39.2	2.29	2.03	4.55	4.60	−	2.30
18	74	39.0	38.4	1.95	1.40	4.37	4.86	−	1.83
19	35	38.6	38.3	2.02	1.91	4.54	4.62	−	0.50
20	6	38.6	38.6	2.07	2.07	4.38	4.62	−	trace
21	17	38.6	38.1	2.47	2.38	4.74	4.86	−	0.30
23	13	38.6	39.5	2.37	2.31	4.88	4.80	−	trace
24	61	39.0	38.6	2.27	2.13	5.30	4.90	−	3.15
26	24	38.4	38.8	2.02	1.38	4.82	4.86	−	1.97
30	175	38.0	38.6	1.64	2.01	4.82	4.88	+	3.90
31	42	39.2	38.7	1.73	1.72	4.65	4.88	−	1.75
33	52	39.4	39.2	2.30	1.90	4.66	5.00	−	1.82
34	187	39.8	39.0	1.81	2.75	5.02	4.87	−	1.79
35	70	39.2	39.2	1.37	1.50	5.14	5.02	+	3.38
36	175	39.0	38.8	1.63	2.22	5.06	4.88	+	4.05
37	27	38.8	38.8	1.34	1.15	4.82	4.60	+	1.20
38	45	38.8	38.8	1.61	1.70	4.82	4.60	+	1.85
40	70	39.0	39.0	2.46	2.54	4.87	4.80	+	2.68
41	25	38.8	38.8	1.45	1.00	4.68	4.64	−	1.15
50[2]	36	38.9	39.0	3.42	3.33	5.45	5.04	+	1.54
75[2]	35	38.6	38.8	2.65	2.34	5.58	5.76	−	1.72
76	24	39.0	39.0	1.88	2.00	5.10	4.71	+	1.38
77	45	39.0	39.2	2.86	2.90	5.77	5.12	−	1.78

[1] + indicates stippled cells found; − indicates no stippled cells found.
[2] These rabbits showed tremors and spasms before death.
[3] This animal became completely paralyzed several days before death.

Experiments on Guinea-Pigs:

Ten pigs were used in this part of the experiment. They received 0.1 c.c. of the 1,000 to 1 mixture on their unbroken skin daily until death (see Table 2). The number of exposures varied from 2 to 46. The average initial weight was 0.449 kilos and the average final weight 0.394 kilos. The average lead per kilo of body weight was found to be 0.855 mg. Three pigs had convulsions and tremors before death.

TABLE 2.—RESULTS OF EXPERIMENTS ON TEN GUINEA-PIGS TREATED WITH ETHYL GASOLINE

(*Dosage: 0.1 c.c. of 1,000 to 1 mixture*)

NUMBER OF ANIMAL	NUMBER OF DOSES	WEIGHT		LEAD PER KILO OF BODY WEIGHT
		Initial	Final	
		kilos	*kilos*	*mg.*
8	26	0.315	0.290	1.10
9[1]	23	0.330	0.290	1.25
10	30	0.367	0.324	1.50
11[2]	4	0.352	0.332	trace
12[2]	2	0.298	0.310	trace
13	23	0.267	0.260	1.20
14[1]	18	0.587	0.545	1.00
15	8	0.637	0.632	0.00
16[1]	46	0.715	0.340	1.75
17	10	0.620	0.620	0.75

[1] These animals had tremors and convulsions before death.

[2] These animals were sacrificed to see if any lead was being absorbed at the time of the trouble at the Bayway Plant.

Experiments on Goats:

Seven goats were given 2 c.c. of the 1,000 to 1 mixture daily by skin application (see Table 3). Their exposures have varied from 86 to 114. Two of these goats have aborted and an analysis of the fetuses showed that they contained an average of 3.45 mg. of lead. Stippled cells were not found in the blood for over two months after the exposures began but they have increased in numbers as time went on. One goat which had received 114 doses of the dilute solution showed a marked muscular weakness in the hind quarters. One other goat showed the same effect to a slight degree.

Two goats were given 1 c.c. of the concentrated tetraethyl lead daily for

TABLE 3.—RESULTS OF EXPERIMENTS ON GOATS TREATED WITH ETHYL GASOLINE

(*Dosage: 2 c.c. of 1,000 to 1 mixture*)

NUMBER OF ANIMAL	NUMBER OF DOSES	STIPPLING[1]	REMARKS[2]
88	114	+	Daily rate of excretion 0.1 mg. The animals are still alive.
89	86	+	
90	86	+	
91	86	+	
92	87	+	
93	86	+	
94	86	−	
(*Dosage: 1 c.c. of concentrated tetraethyl lead*)			
95	60	−	Daily rate of excretion 0.56 mg.
4016	59	+	

[1] + indicates stippled cells found; − indicates no stippled cells found. Stippling did not begin to show in these animals until they had received a number of doses.

[2] Goat 88 showed weakness in the hind quarters and was very nervous. Goats 88, 93, 95, and 4016 each aborted.

Goat 93 showed weakness especially in the left hind leg.

Goats 95 and 4016 were very quiet and stood hunched up at times as if they had colic.

approximately two months. Both of these goats have aborted. One died after receiving 60 exposures. It had very marked weakness in the hind quarters for several days before it died. The day that it died it was unable to stand or to use its hind limbs, and spent the whole day crying. An analysis of the various organs showed the lead to be distributed in the following manner:

	Weight	*Lead Content*
	gm.	*mg.*
Brain	60	3.36
Lungs	250	1.74
Kidney	75	0.80
Liver	250	1.85
Heart	125	2.93
Muscle	...	0.67[2]
Bone	...	4.86[2]
Intestines	not taken	3.27

Both of the goats receiving the concentrated lead compound remained quiet and hunched up as if in pain most of the time.

The goats being treated with the 1,000 to 1 mixture excreted an average of 0.1 mg. of lead each twenty-four hours. The goats receiving the concentrated tetraethyl lead excreted an average of 0.56 mg. each twenty-four hours.

Fume Hazard Tests

At the very beginning of our work we realized that it would be hard to duplicate the actual conditions that may be present around a filling station or a garage. No data were available as to the concentration of gasoline that may occur at such localities. These data would have been very valuable to us in planning our work.

[2] Per 100 gm.

Numerous inquiries at filling stations seem to indicate that, if a man were careful, the spillage around a pump should not amount to more than 1½ gallons a day. But even if this amount is correct, the concentration in the immediate atmosphere would vary from time to time, depending on the area over which the gasoline spread itself, on the temperature, and on the air movements. Again it does not necessarily follow that the man at the station is exposed to the fumes for more than a few minutes at a time or for many minutes a day. In considering this hazard one must bear in mind that R. P. Albaugh[3] has shown that when a man is exposed to a mixture of air and benzene 80 per cent. of the benzene is absorbed. The same thing may be expected to hold true for a mixture of air and gasoline. If this gasoline should contain lead, it would be a most excellent method for getting the metal into the body through the lungs.

Through Evaporation in a Gas Chamber

In our study of the ethyl gasoline fumes we made use of a gas chamber containing approximately 180 cubic feet of air. The air in this chamber is changed once in ten minutes or 6 times an hour by means of an air inlet near the floor of the chamber and an outlet in the ceiling. The ethyl gasoline fumes were admitted with the incoming air in sufficient quantity to maintain a predetermined dilution of gasoline fumes in the chamber. This was accomplished by bubbling air through an ethyl gasoline mixture in a

[3] Albaugh, R. P.: Benzene Poisoning. Mod. Med., 1919, *1*, 670; abstr. in Chem. Abstr., 1920, *14*, 575.

bottle at the proper rate. We did not evaporate all of the gasoline in the bottle because it has been observed that there is a concentration of the tetraethyl lead as the lighter portions of the gasoline are evaporated. For example, we found that we would have no difficulty in evaporating 400 c.c. of ethyl gasoline spilled on nonporous material under out-of-door conditions in four hours. Four samples of 400 c.c. of gasoline were evaporated in shallow glass dishes under natural conditions out of doors. Some of the tetraethyl lead in the gasoline decomposed and the yellow crystals of hydrated triethyl lead salt were formed. An analysis of the four residues showed that they contained on the average 0.108 gm. of lead. The original 400 c.c. contained 0.372 gm. of lead. This is what one might expect under industrial conditions, and the man would also have some exposure to these fine crystals of the lead salt as well as to the ethyl gasoline fumes.

In our work we endeavored to evaporate 400 c.c. of the 600 c.c. of gasoline that the bottle would contain during the four hours that the animals were exposed in the gas chamber. As far as we could determine, the air of the chamber during our experiments contained 0.04 mg. of lead per cubic foot.

Before we exposed our animals to ethyl gasoline fumes of this concentration, we exposed them to the same concentration of gasoline fumes which did not contain lead for twenty hours a day for one week. At the end of this preliminary period, the animals showed no ill effects and we felt that our predetermined concentration of gasoline in the ethyl gasoline fumes would not injure them in any way when they were exposed for only four hours a day. Any ill effects that might be observed in the animals we felt sure must be ascribed to the lead in the ethyl gasoline fumes.

Animal Experiments:

Goats, rabbits, guinea-pigs, monkeys, pigeons, and rats were exposed in the gas chamber, for an average of four hours a day, to the fumes of ethyl gasoline of the aforestated concentration. Care was taken that no food or other material that might be gnawed was present. The only way that lead could have entered the body was through the respiratory tract unless some vapor should have been deposited on their fur, which is highly improbable. Strangely enough, our albino rats seemed more sensitive to the fumes than the rest of our experimental animals. The total number of hours that the animals were under exposure varied from 30 to 265 (see Table 4). As before, we do not claim that lead was the cause of death, especially in the cases of those animals that died after a short exposure. Smears of the goats' and rabbits' blood did not reveal any stippled cells when the exposure began, but as the number of hours of exposure increased, stippled cells were observed in every case. The animal usually became quiet and hunched up for several days before it died. As in the cases of the men at the Reconstruction Hospital and of the animals dying after skin exposures, the autopsies gave no special findings. An analysis of the carcasses of thirty-nine animals that died showed that they contained on an average 5.52 mg. of lead per kilo of body weight.

TABLE 4.—RESULTS OF EXPERIMENTS ON ANIMALS EXPOSED TO FUMES RESULTING FROM EVAPORATION OF ETHYL GASOLINE IN A GAS CHAMBER

ANIMAL	FINAL WEIGHT	HOURS EXPOSED	LEAD PER KILO OF BODY WEIGHT
	kilos		*mg.*
Monkey	3.200	30	0.075
Guinea-pig	0.552	56	0.235
Monkey	3.000	56	0.166
Guinea-pig	0.352	70	2.272
Guinea-pig	0.365	70	2.328
Guinea-pig	0.507	70	1.341
Guinea-pig	0.540	70	1.666
Rat	0.128	88	7.812
Guinea-pig	0.480	96	0.625
Pigeon	0.280	96	1.071
Rat	0.135	96	5.925
Goat	13.200	100	0.015
Rat	0.119	100	6.720
Goat	14.500	114	0.068
Rat	0.141	148	12.056
Rat	0.123	150	16.016
Rat	0.138	152	6.884
Rat	0.142	152	7.464
Guinea-pig	0.365	167	5.068
Rat	0.140	167	11.000
Rat	0.138	174	10.652
Rabbit	1.660	175	1.927
Guinea-pig	0.410	176	3.487
Guinea-pig	0.348	178	2.298
Guinea-pig	0.367	178	3.269
Guinea-pig	0.595	178	1.848
Rat	0.122	179	9.016
Rat	0.125	188	12.000
Rabbit	2.040	193	1.259
Rat	0.147	210	11.224
Rat	0.135	224	7.333
Rat	0.152	238	6.644
Rat	0.124	241	8.145
Rat	0.128	254	7.812
Rat	0.137	258	7.299
Rabbit	2.520	258	1.765
Rat	0.130	260	14.460
Rat	0.157	260	12.547
Guinea-pig	0.480	265	3.479

One of the guinea-pigs gave birth to two pigs. Neither the mother nor her young were exposed to lead after their birth. The two young pigs lived seven days, then had convulsions and died. An analysis showed that their bodies contained 0.6 and 0.68 mg. of lead, respectively.

Lead was found in the excreta of all the animals exposed. None of the control animals showed lead when their carcasses were burned and analyzed.

Through Natural Evaporation

Test 1.—This experiment took place on the roof of the building where the rate of the vaporization depended on the natural air movements and temperature during the day and night. The fumes were not confined and could disperse into the surrounding atmosphere according to the ordinary physical laws.

For this work we took a galvanized iron pan 30 inches in diameter and 20 inches deep. The bottom of this container was flat. A movable false bottom of one-half-inch mesh screen was placed 11 inches above the bottom. Each morning we poured 1,800 c.c. of ethyl gasoline on the bottom of the pan after it had been thoroughly cleaned and dried. The movable false bottom was then placed in position and on it were placed ten albino rats. The top of the pan was left open to the natural air movements, except for one-half-inch screen placed on the top to keep the rats from jumping out. The rats were exposed to the fumes resulting from the natural evaporation of the tetraethyl gasoline for twenty-two hours daily for seven days. For two hours each day, the rats were removed to give them a chance to eat and drink,

and to permit the cleaning of the container. It was observed that approximately 200 c.c. of gasoline were left unevaporated at the end of the twenty-two hours. At the end of the seven days, the rats were killed and examined. An average lead content of 26.6 mg. per kilo of body weight was found. There was no way that the rats could have come in contact with any lead except through the fumes formed by the natural evaporation of the ethyl gasoline. There was no dust, as the bottom of the pan was still moist. We feel after this experiment that there can be no doubt in our own minds that the lead concentration in the ethyl gasoline fumes is of sufficient degree to cause lead storage in the body in appreciable quantity.

In reporting this test, we do not claim that man has the same exposure that these rats had. That the exposure of the animals was not unduly severe, however, is plainly indicated by the fact that they did not exhibit any distress from the gasoline fumes, but acted in a perfectly normal manner during the whole day whenever they were observed. Of course no man will be exposed to fumes for twenty-two hours a day continuously, nor will he be standing over a pool of gasoline.

Test 2.—In order further to study the hazard of ethyl gasoline fumes we erected a pen 10 feet long, 4 feet wide, and $3\frac{1}{2}$ feet high in a large yard in a neighboring suburb. We placed a roof over the pen to shut out the rain. This roof was not very tight, however, because of cracks between the boards which permitted the escape of fumes. Three sides of the pen consisted of wire screening and the fourth side was formed by the side of a building. Any prevailing winds could sweep through the pen. The floor was made of loose stones covered with dirt. This dirt was removed from the floor every so often so as to keep the pen sanitary. About 18 inches from the open end of the pen a circular enclosure of wire netting having a diameter of 12 inches was erected and extended from the ground nearly to the roof. The bottom of this enclosure was hollowed out to a depth of 6 inches below the surface of the surrounding floor. A bucket containing ethyl gasoline was suspended, in this circular enclosure, from the roof. In this bucket were placed two lamp wicks which hung over the sides of the bucket and rested on two boards fastened together in the shape of a V. The point of this V rested on a plate in the bottom of this enclosure. The wicks permitted a slow flow of ethyl gasoline, by means of capillary attraction, from the can on to these boards. The gasoline evaporated as it flowed down the boards. Any gasoline that did not evaporate was collected in the plate and any overflow from the plate had the opportunity to soak through the loose stones into the ground. The floor of the pen did not get wet from any gasoline so far as our observations went. The bucket was loosely covered so as not to interfere with the capillary flow. We found that we could evaporate about 2 quarts of gasoline each twenty-four hours.

Fifteen rabbits were placed in this pen and were constantly exposed to any fumes that might be present in the

atmosphere. These rabbits had the freedom of the pen and could run around at will. But under no circumstances could they come in contact with any gasoline fluid. Food, which was given them twice a day, was quickly eaten. In carrying out this experiment, we felt that we were exposing the animals under more natural conditions than in some of our other work. The animals were active and showed no signs of being under any exposure. In fact anyone seeing them would say that they were in excellent condition. They all gained weight.

We did not analyze their feces nor make any blood examination as we were principally interested in learning whether or not the rabbits under these conditions would absorb lead. Visitors often remarked that the gasoline fumes were no more in evidence than around filling stations, if as much.

After these animals had been constantly exposed from June 6 to July 17, five of the animals were killed to see if any storage of lead had taken place within that time. A chemical analysis showed that they had stored an average of 1.72 mg. of lead per kilo of body weight. This experiment confirmed our previous observation as to the ease with which the lead can be absorbed by the organism from the air-gasoline mixture. We did not determine the concentration of the gasoline in the air because it varied so much with the temperature and the air movements. During the period between June 6 and July 17 we had some of the hottest weather of the year; we had also considerable cloudy weather and rain with high winds.

Possible Hazard from Contact with Tetraethyl Lead

The few experiments noted below will indicate the possible hazard from any concentrated tetraethyl lead that might be present through a concentration of the gasoline by vaporization. This hazard is also one of those with which the manufacturer has to contend.

Rabbits and guinea-pigs were used in these experiments; 0.01 c.c. of the concentrated ethyl lead compound was allowed to flow from a small pipette on to the skin of the animal daily until death ensued.

Summary of Experiments on Five Rabbits

The animals received from 13 to 19 doses before dying. The average findings are as follows:

	Temperature °C.	*Weight* kilos	*Red Blood Count* per c. mm.
Initial.....	38.7	2.2445	4.77
Final.....	39.8	2.2040	4.71

An average of 1.79 mg. of lead was found in the viscera of the rabbits (see Table 5). Stippled cells were found in two of the rabbits which received 18 and 19 doses. All of the rabbits became quiet a couple of days before they died. This quietness was succeeded by tremors, exaggerated muscular movements, and, finally, convulsions.

A histologic examination of the various organs gave no indication of any change that we could say came from the tetraethyl lead exposure. Brain lesions were observed but the same lesions are noticed in 50 per cent. of the normal animals examined.

Summary of Experiments on Five Guinea-Pigs

The pigs received from 8 to 20 doses before dying. The average initial weight was 0.345 kilos; the average final weight, 0.337 kilos (see Table 6).

An average of 4.93 mg. of lead per kilo of body weight was found in the whole carcass. The pigs all developed tremors and convulsions before death. The autopsy findings revealed nothing that was not found in control animals.

TABLE 5.—RESULTS OF EXPERIMENTS ON FIVE RABBITS SUBJECTED TO CONTACT WITH TETRAETHYL LEAD

(*Dosage: 0.01 c.c. of concentrated tetraethyl lead*)

NUMBER OF ANIMAL	NUMBER OF DOSES	TEMPERATURE		WEIGHT		RED BLOOD COUNT		STIPPLING[1]	LEAD IN VISCERA
		Initial	Final	Initial	Final	Initial	Final		
		°C.	°C.	kilos	kilos	per c. mm.	per c. mm.		mg.
1[2]	16	38.9	39.2	1.600	1.370	4.51	4.68	−	2.25
2[3]	19	39.0	38.4	3.100	3.060	4.90	4.62	+	no analysis made
3[2]	18	38.8	40.8	2.760	2.210	4.92	4.74	+	1.66
5[2]	13	37.6	38.4	2.112	1.840	4.82	4.82	−	1.73
6[2]	18	39.6	42.2	2.655	2.540	4.69	4.72	−	1.52

[1] + indicates stippled cells found; − indicates no stippled cells found.

[2] These rabbits became quiet a couple of days before their death. This quietness was succeeded by tremors, exaggerated muscular movements, and, finally, convulsions.

[3] This rabbit was sent to Presbyterian Hospital for a special histologic examination.

Control Animals

We have not detected lead in any of our control animals, as care was taken never to have them under exposure.

General Remarks

In rendering this report we realize that it is very difficult to apply animal experiments to human beings. Still we feel that our findings so far indicate a potential hazard in the use of ethyl gasoline by the public without some educational campaign. While man is not confined in the same way that the experimental animal is, and can walk in and out of the experiment, does this fact only mean that it will take a longer time before any pathologic condition may arise? The question as to what constitutes lead poisoning has to be definitely decided by the medical profession in the near future.

"Looney Gas" poisoning is a wrong terminology, and it is to be regretted that the newspapers were permitted to coin this term. Both from our own work and from that of Harnack on triethyl lead, we are inclined to feel that all of the cases on record can be described as cases of lead poisoning, either acute or chronic. Harnack showed in his work that the triethyl lead acted as the whole molecule on the circulatory and respiratory centers for a short period. If the animal survived this period, the compound broke down and an inorganic lead compound was formed in the body and all of the

secondary symptoms were those of inorganic lead poisoning. He was unable to find any of the original molecule in the bladder. We have made three or four attempts to detect tetraethyl lead in the animal several hours after an exposure to concentrated tetraethyl lead, but our results were always negative.

TABLE 6.—RESULTS OF EXPERIMENTS ON FIVE GUINEA-PIGS SUBJECTED TO CONTACT WITH TETRAETHYL LEAD[1]

(Dosage: 0.01 c.c. of concentrated tetraethyl lead)

NUMBER OF ANIMAL	NUMBER OF DOSES	WEIGHT		LEAD PER KILO OF BODY WEIGHT
		Initial	Final	
		kilos	*kilos*	*mg.*
3	8	0.400	0.410	4.268
4	14	0.352	0.357	7.002
5	4	0.242	0.200	trace
6	13	0.370	0.344	6.104
7	20	0.360	0.377	7.294

[1] All developed tremors and convulsions before death. The autopsy findings revealed nothing.

Human Observation

Two persons who have followed the work since the beginning and who have taken precautions, knowing the poisonous nature of the compound, have stored lead as shown by an examination of the urine and feces:

In Feces	*In Urine*
mg.	*mg.*
0.76 in 24-hr. sample	0.08 in 200 c.c.
	0.14 in 200 c.c.
0.42 in 24-hr. sample	0.07 in 100 c.c.

It is interesting to observe that neither one of these persons noted any special symptoms, and in fact one of them had passed two insurance examinations within a month. They both spoke of wakefulness at night, irrational dreams, nervousness, and a general feeling of malaise which expresses itself in a general lack of "pep." Any doctor treating them and not knowing their exposure would have ascribed it to a run-down condition, needing a little tonic, etc.

Conclusions

Our experiments indicate apparently that there is no doubt that animals exposed to skin application of ethyl gasoline will store lead; also that the rate of excretion does not equal the rate of absorption. This is entirely in agreement with all medical experience. We are not prepared to say that all of the animals that died did so because of the lead. We have, however, noticed at various times that if for any reason we do not expose the animal which may appear sick for several days, the animal will show a marked improvement. This is again in line with medical experience in cases where patients have been exposed to lead. One cannot but feel, on account of this observation, that their condition before the rest was due to lead exposure. This may account for the fact that so far no cases of lead poisoning have been found at filling stations or in garages. The time between the man's exposures may be long enough to permit him to remain in apparent good health though he may be storing lead. It may take years before he stores enough or before a sufficient change takes place in his metabolism to cause the symptoms commonly attributed to lead poisoning. The fact to bear in mind is that

there is no doubt that a storage of lead takes place under exposure. Lead stored in the body constitutes a potential lead poisoning.

The same observations that have been made about animals receiving skin applications can be made on the animals that were exposed to fumes of the dilute ethyl gasoline. It is of significance that there is a continuous storage of lead taking place in the animals no matter whether it is by absorption through the skin or by inhalation. What is of special significance, or what seems so to us, is the fact that the animals in the gas chamber seem to show the effects more quickly than those which received skin applications. The dilution does not seem to make any difference except for the longer time necessary to accumulate the same quantity of lead.

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A Mobile Refrigerating Unit[1]

By H. F. Pierce

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THE advent of the small, electrically operated refrigerating plant, designed primarily for use in the home, has provided hospitals and laboratories with a convenient means of chilling perishable materials, and of maintaining conditions of low temperature either for storage or for the performance of certain types of experiments.

Such a refrigerating unit consists essentially of a motor-driven compressor pump, a cooling coil, an expansion valve, and an expansion coil located in a brine tank. The thermostatic control provided consists of a metal bellows (sylphon tube[2]) containing a volatile liquid which by its expansion and contraction actuates the starting switch of the compressor motor. When the brine temperature falls the motor stops; when it rises the motor starts again, thus making the unit automatic in operation. The brine tank is designed to occupy the ice compartment of the usual household refrigerator.

In order to extend its field of usefulness, Prof. Ernest L. Scott, of this department, conceived the idea of mounting the refrigerating unit on a low truck so that it might be easily moved about the laboratory; of placing on the same base a cooling chamber in which materials could be stored and certain experiments performed; and of connecting to the brine tank a motor-driven pump which should serve the double purpose of keeping the cold brine in circulation through the unit and of pumping it through any outside apparatus in which it might be desirable to maintain a low temperature. The work of constructing this mobile unit was begun under the direction of Professor Scott and completed by the author.

Figure 1 shows the apparatus and the disposition of its various parts. The brine tank, *C*, is insulated on the sides and bottom with 4 inches of sheet cork and on the top with 6 inches of granulated cork, and the whole is inclosed in a wooden casing which serves to protect the insulation and to support the relays, switches, and other control devices. The rotary pump, *D*, which circulates the cold brine through the cooling chamber, *E*, and any outside apparatus attached to *F* and *G*, is, with the regulating valves, housed in a metal box filled with granulated cork. The cooling chamber is an annular copper tank covered with compressed cork insulation. It rests on a sheet cork base and is provided with a movable

[1] Received October 15, 1925

[2] Griffiths and Awbery, *J. Sci. Instruments*, 2, No. 11, 368 (1925).

cover of the same material. Its depth is 14 inches, its diameter 10 inches. A small fan in the chamber keeps the air in circulation and assists in maintaining an even temperature.

Since the sulfur dioxide thermostat supplied with the unit allowed a variation in brine temperature of some 10° C., it was decided to build a more sensitive control device. Prof. H. B. Williams, of this department, suggested the use of a large mercury thermostat, the mercury to be contained in a steel tube and the usual glass capillary tube to be attached to it by means of a perforated rubber stopper. This suggestion was adopted, and the mercury thermostat, used in connection with the control system devised by the author,

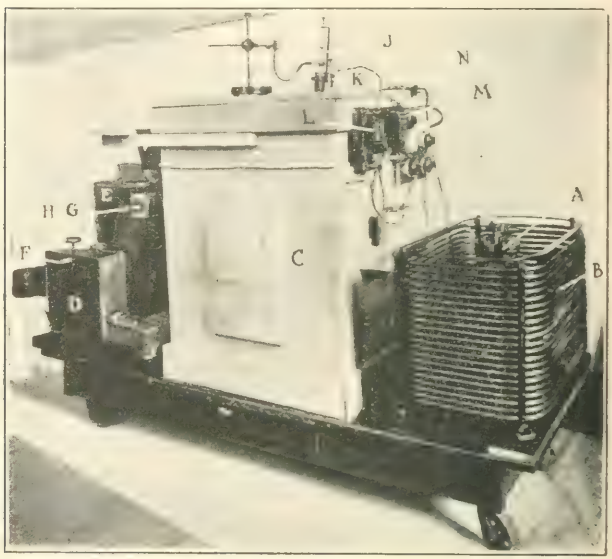

Figure 1—Mobile Refrigerating Unit

A, compressor pump; *B*, cooling coil; *C*, brine tank; *D*, brine circulating pump; *E*, cooling chamber; *F* and *G*, outlet and inlet for cold brine supplied to outside apparatus; *H*, controlling valves; *J*, mercury thermostat; *K*, resistance shunted across mercury contact; *L*, damped telegraph relay; *M*, contactor relay; *N*, plug for attaching unit to electric mains

has given entire satisfaction. The steel tube is 14 inches long and 0.5 inch in internal diameter, and is plated with tin outside to prevent corrosion. During the process of filling, the mercury was boiled in the tube to eliminate air bubbles. The tube is provided with a threaded brass head which makes a tight joint with an opening in the top of the brine tank, and to this head is attached a brass tube which is concentric with and furnishes support and protection for the glass tube piercing the rubber stopper. Near the upper end of the glass tube is a 2-way cock, which allows the thermostat tube to be connected with the mercury reservoir. Attached to the upper end of the brass tube but in-

sulated electrically from it is an arm carrying the nickel wire which makes contact with the mercury in the capillary tube.

The mercury thermostat is connected through three standard dry cells with a telegraph relay, *L*. In order to minimize sparking at the surface of the mercury, a high resistance, *K*, is shunted across the gap. A "variable grid-leak," 0 to 50,000 ohms, as used in radio receiving apparatus, serves admirably for this purpose.

Since the motor requires a larger amount of current than can be carried safely by the telegraph relay, a contactor relay, *M*, is employed. This is a commercial device intended for the remote control of motors, heating devices, or large groups of lamps, and is so designed that in closing it completes two circuits, one supplying the motor or other device, the other maintaining the flow of current through the relay and keeping it closed until its coil is short-circuited by some outside connection. This relay will safely break a current of 15 amperes at a pressure of 125 volts, and requires only 0.027 ampere for its operation.

The telegraph relay was modified by the addition of a second insulated contact, so that the armature makes an electrical connection at each end of its stroke. When the temperature of the brine rises, the mercury mounts in the capillary tube and touches the nickel wire, the telegraph relay closes, the contactor relay closes, and current flows to the motor. When the operation of the unit has reduced the brine temperature so that the mercury falls, the telegraph relay opens and makes a second contact. This short-circuits the contactor relay coil through the resistance *R*, the contactor relay opens, and the motor stops.

Owing to vibration caused by the motors and pumps and consequent repeated momentary contacts at the mercury surface, the light armature of the telegraph relay had a tendency to flutter back and forth between its contacts. This tendency was overcome by attaching to the armature a short rod carrying a ball which fits closely in a cylindrical brass cup filled with heavy oil (*T*, Figure 2). Thus damped, the relay requires approximately 30 seconds to close and an equal length of time to open. Hence, in spite of vibration at the mercury surface, there is no chattering of either relay, and the motor starts and stops smoothly. Damping the relay naturally interferes somewhat with the sensitivity of the control, but it is necessary because of the wear and tear which would otherwise accrue with more frequent starting and stopping of the motor.

The accuracy of the thermostatic control is very good, considering the fact that the temperature is regulated by controlling the compressor motor. A Beckmann thermometer placed (in air) in the cooling chamber showed a deviation from the mean of $\pm 0.075°$ C. over a period of 4 hours at a temperature of approximately 0° C., with a room temperature varying between 17° and 22° C. With marked

changes in room temperature a somewhat larger deviation has been observed, but in all cases the temperature has remained within $\pm 0.25°$ C. over periods ranging from 1 day to 2 weeks.

In place of the usual calcium chloride brine we are using a 35 per cent alcohol-water mixture. This fluid is satisfactory for temperatures as low as $-15°$ C., and it has a

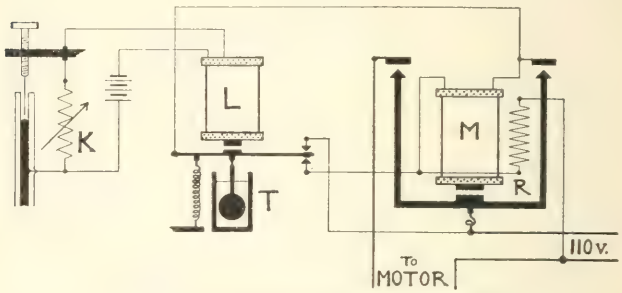

Figure 2—Thermostatic Control Apparatus

refrigerating capacity nearly equal to that of a 25 per cent calcium chloride solution.[3] It has the advantage of being noncorrosive to the metal parts of the system and of leaving no hygroscopic deposit in case any is spilt.

This apparatus has been in nearly constant use during the greater part of a year, and has required little attention other than cleaning and oiling.

[3] Zoller, THIS JOURNAL, **16**, 1073 (1924).

THE EINTHOVEN STRING GALVANOMETER, A THEORETICAL AND EXPERIMENTAL STUDY

PART 2

By Horatio B. Williams

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PART 2[1,2]

SECTION 1. GENERAL PRINCIPLES OF CONSTRUCTION

Certain of the operating characteristics of the string galvanometer, such as short period, good damping, stability of zero under constant string tension, are common to all instruments of this type however crudely they may be made. Others, such as (a) stability of zero and of the plane of deflection under varying degrees of string tension, (b) equality of successive deflections for successive equal increments of current through the string, (c) capability of adjustment to very great sensitivity, (d) freedom from disturbance by extraneous vibration, (e) sharpness of optical definition, (f) amount of transmitted light available for photographic registration, etc., are distinctly dependent on design and construction. These matters concern the prospective user as well as the designer and maker, and an understanding of them is necessary if one is to make an intelligent choice from a number of available types of an instrument suited to fulfill a given requirement. Essential parts of all string galvanometers are the magnet, the string mounting, the microscopes and the string. Before the special problems

[1] The theory of the string galvanometer is treated in Part 1, this Journal, *9*, pp. 129–74, August, 1924. I am indebted to Professor H. W. March, of the Department of Mathematics, University of Wisconsin, for bringing to my attention inconsistencies in the statement of the solutions (10) and (16) of Part 1. The correct statement of these solutions is given in Appendix A, Part 2, where will also be found corrections of other errata in Part 1.

[2] When Part 1 was written the writer was unaware of a treatment of the theory of the Einthoven galvanometer published in English in 1914 by Dr. L. S. Ornstein. See Section VII, Part 2, for comment on this paper.

of string galvanometer design can be appreciated it is necessary to have some familiarity with these parts and their functions and we shall proceed to discuss their more general features.

A. THE MAGNET

1. *General Remarks.* The string galvanometer may be regarded as a special form of moving coil instrument and it was actually from this standpoint that it was developed [1]. The strength of the magnetic fields is one of the factors which determines the sensitivity of a moving coil galvanometer, but it is well known that the sensitivity of the ordinary type of moving coil instrument cannot be increased indefinitely by augmenting the strength of the fixed field. It is a matter of great difficulty to make a coil without contamination of the wire or insulation or both with paramagnetic material and unless the distribution of this material is uniform and the field homogeneous, there will result an actual diminution of sensitivity when the field strength exceeds a rather moderate value. The moving system of the string galvanometer, consisting of a single tenuous conductor with no insulating covering, is less liable to accidental contamination. The strings are made of material which initially is not likely to contain significant traces of magnetic material. It is customary to employ field strengths of 20,000 gauss and more. The moving coil galvanometer can be made more sensitive by reducing the elastic restoring couple of its suspension, but a rather definite limit is set by the difficulty of maintaining a stable zero when the restoring force is made very small. It is usual to attain greater sensitivity by increasing the number of turns in the moving coil. In the string galvanometer this means of increasing sensitivity has been deliberately foregone and the coil replaced by a single stretched conductor in order to secure a very short natural period. The possibility of employing a field as strong as 20,000 gauss is of some assistance in regaining sensitivity, but the gain from this source corresponds to the use in a D'Arsonval type of a coil of ten turns in a field of 1000 gauss. That the string galvanometer can be at once sensitive and of short period is possible because the inertia of the moving part is very small and a small restoring force will suffice. Also *the zero position of a stretched string is very stable even when the tension is small.*

Although the field should be strong, it is usually not advisable to attempt to reach such field strengths as have been attained with large electromagnets in recent years. These magnets are designed to be excited with powerful currents and a slight rise in temperature is

usually immaterial. Sometimes they are wound with insulated copper tubing for continuous water-cooling and the amount of energy dissipated may be very large. In a measuring instrument precision must take precedence over sensitivity. The strings of a string galvanometer are often made of material whose coefficient of expansion differs greatly from that of the supporting framework and change in temperature of the instrument will affect its sensitivity. The field strengths which may be attained without great initial expense and the dissipation of large amounts of energy in the field coils are so large that they could hardly be doubled by the most elaborate and expensive construction. It is certainly not worth while to multiply the cost of construction and operation many fold for a twofold increase of sensitivity.

The desirability of concentrating the windings in the neighborhood of the airgap when intense fields are to be produced is now well known (2), (3). On the other hand if the poles are large the concentrated winding makes a coil whose average length per turn is great. The magnetizing effect is proportional to current and turns, the energy expenditure to resistance multiplied by current squared. Fields of very considerable intensity may be produced with comparatively small energy expenditure by distributed windings if the design of the iron frame is such that the permeability is high when the flux density is sufficient to maintain the desired flux in the gap. The only drawback is the somewhat extensive stray field which may affect other instruments.

2. *Lateral Uniformity of Field.* It is evident from the theory that the field must be uniform in the direction of the string's motion if the deflections are to be proportional to the strength of the current flowing in the string. As the string galvanometer is used almost entirely as a deflection instrument it is highly desirable that the law of deflection should be a linear one. The necessary uniformity can be secured by care in making the pole faces approximately parallel plane surfaces. The accuracy necessary to secure proportionality over more than a very limited range is really considerable. Failure to meet this requirement is one of the most common faults in commercial string galvanometers. Einthoven's original instrument, which we shall have occasion to mention frequently and shall hereafter refer to as the Leyden Model, gives deflections proportional to the strength of current for ten centimeters either side of zero with a magnification of 660 diameters and at a current sensitivity of about 10^{-7} ampere/cm. The replica of this instrument in the physiological laboratory of Columbia University

gives a similar result, but with a magnification of 900 diameters. Most commercial instruments have a much smaller range of linear proportionality.

3. *The Perforation for the Optical System.* This should be no larger than necessary, though its effect has been somewhat overestimated. As remarked by DuBois, [4], a considerable internal leakage of flux from the edges of the opening and the field in this central region may be of considerable intensity. The size of the perforation is determined by the limitations of the lens maker's art. The lenses must be of high aperture, but it is not necessary that the pencil of light should be as large as that transmitted by a standard microscope objective of the same aperture. It would be possible theoretically to reduce the diameter of the lens system very materially, but this would entail expensive changes which the manufacturing optician could probably not afford to make considering the limited market for them. Up to the present time the makers of string galvanometers have contented themselves with standard microscope objectives mounted in the smallest metal case which would accommodate them. The effect of the perforation will be discussed in detail in Section IV of the present paper.

4. *The Length of the Airgap in the Direction of the Lines of Force.* In the Leyden Model this dimension is two millimeters and in most of the commercial models it has been the same or greater. The principal reluctance in the path of the flux is this airgap. It is much the largest factor in determining the necessary magnetomotive force and thus in great measure determines the size of the magnet. The weight of the Leyden Model is 100 kilos. For some purposes lighter instruments are desirable. One of the new designs described in section 2 has an airgap of one half mm. There are, however, several disadvantages to be considered in connection with the reduction of the length of the airgap. First the difficulty of securing a linear deflection law is increased. A slight departure from accurate parallelism of the pole faces will have a more marked effect on the distribution of the field. This difficulty is not too great to be overcome by care and mechanical skill. There remains the danger that dust, particularly particles of iron may gain access to the gap and, becoming attached to the pole faces, touch the string and impede its movements or damage its coating. Experience has shown that with reasonable care an instrument with a half mm gap can be operated successfully, but the gap must be carefully inspected each time it has been opened to change strings, etc., to make sure that it is free from foreign matter.

5. *Necessity for Mechanical Rigidity.* There is a not inconsiderable tractive force between the polepieces[3] and this requires a construction of sufficient strength and rigidity to prevent deformation when the field is excited. It is usual to mount the microscopes and the parts that carry the string on the magnet. In connection with the mounting of the string it is necessary to take into account a molecular change, the magnetostrictive effect, which produces a small but significant deformation of the polepieces.[4]

6. *Shape of the Polepieces.* In order to concentrate the field in a narrow space the polepieces are made wedge-shaped. The angle included between the faces of the wedge was 90° in the Leyden Model and this angle has been adopted in many subsequent models. For the polepieces of large electromagnets in the form of truncated cones, Stefan, (5), showed theoretically that an included angle of about 110° is most favorable for producing a field of maximum intensity. DuBois, (4), has confirmed this result experimentally and shown that a difference of a few degrees is not critical. Salomonson (6) concludes that for a prismatic pole of a string galvanometer formed on the end of a circular cylinder 5 cm in diameter a top angle of 110° would be advantageous. DuBois (4) concluded that 90° would give the most intense field for a prismatic pole of infinite length. In the galvanometer later described as Type B this angle was made 120°. This was done as a matter of mechanical convenience and while it may have resulted in sacrificing some intensity, the practical result has been entirely satisfactory. DuBois has shown also that the greatest uniformity of field requires an angle smaller than that for maximum intensity. Uniformity is a prime requisite in the string galvanometer, but is required for only a small fraction of a millimeter in the middle region. Experiment shows that uniformity over the necessary extent can be attained even with the large angle provided the polefaces are made reasonably good approximations to plane parallel surfaces.

B. THE STRING MOUNTING

1. *General Remarks.* The sensitivity and period of the string galvanometer can be altered within wide limits by varying the tension on the string. In making this adjustment it is a great convenience if the string shadow remains in focus and retains the same zero throughout.

[3] The theoretical data for calculation of this tractive force are given by Maxwell, Treatise on Electricity and Magnetism, Edition of 1873, *2*, p. 255. A discussion of Maxwell's law is given by H. DuBois, The Magnetic Circuit, Translation by Dr. Atkinson, p. 167, 1896.

[4] See sub-section B, Article 2, of this Section.

It is possible to secure this condition, the possibility depending partly on the properties of the strings themselves and partly on certain adjustments. Assuming the string suitable,[5] the other necessary condition is that it shall be centered with considerable accuracy in the magnetic field. Though the field can be given uniformity in the direction of the string's motion for something less than a tenth of a millimeter either side of the middle of the poleface and midway between the poles, it will always be more intense close to the pole faces. All strings are either diamagnetic or slightly paramagnetic and by reason of slight contamination parts of a string may be paramagnetic and others diamagnetic. If the string is exactly centered its magnetic properties make little difference in its behavior, but if not it is likely that on deflection it will approach one or other of the pole faces with the result that its shadow will go out of focus. The same thing and probably a change of zero will result if the improperly centered string is slackened. These difficulties are readily avoided if means are provided for accurate centering. In the Leyden Model the clamps which receive the metal tips to which the strings are soldered are fastened to accurate and smoothly working cross-slides actuated by screws. There are two such slides, one at top and one at bottom and they move the one parallel and the other perpendicular to the polefaces. Devices of a less expensive nature have been tried with varying success. The results will never be altogether satisfactory unless the centering devices operate smoothly and precisely. Few instrument makers seem to have realized the need for precision in these devices and those who have never worked with a properly constructed string galvanometer in good adjustment consider the faults of the designer and builder to be inherent in the instrument. This is the more remarkable because the facts were set forth in detail by Einthoven (7, p. 314) in 1909.

2. *Tension Adjustment.* There must also be a fine adjustment for varying the tension on the string and this should operate smoothly and with a minimum of lost motion. When the tension on the string is small, a very small movement of the upper end will produce a relatively large change in tension (7, p. 308), so that an adjustment capable of close setting is desirable. An adjustment of the same general character as the wormgear fine focussing adjustments supplied on the best makes of microscopes is entirely satisfactory. A fine adjustment

[5] See sub-section D of this section, Article 4.

obtained from the Zeiss Works, of the type found on their large microscope stands, was used for the string tension adjustment on the Columbia instrument and has met every requirement.

3. *String Mountings.* There are two principal methods in use for mounting strings. The method employed in the Leyden Model in which the mountings are fastened permanently to the magnet and the strings inserted directly into the instrument is simple, substantial and effective, but requires of the operator considerable manipulative skill when strings require to be replaced. This type of mounting is entirely satisfactory for laboratory use where it will be handled by skilled personnel. In the other type the strings are mounted in a removable member carrying all or part of the centering and tension adjustments. This member often takes the form of a case in which the string is completely enclosed. The second plan presents itself in a considerable variety of designs. It offers obvious advantages when properly designed and executed, but on account of the difficulty of providing a number of precise adjustments in a compact removable member, it is particularly in instruments of this type that highly "simplified" adjustments are often found. It is essential that whatever plan is adopted shall secure practically dust-tight enclosure of the string when it is finally mounted and protect it against disturbance by air currents. Einthoven covered his entire instrument with a large box. This plan permits great simplicity in the construction of the instrument and has few disadvantages if, as is usual, the instrument is to be kept and used in one place. The same arrangement was adopted in the Columbia instrument with satisfactory results. If equally precise and robust adjustments can be provided in a construction which gives adequate protection to the string without requiring the covering of the entire instrument, it makes for somewhat greater facility in changing strings and permits freer radiation and convection of heat from the magnet coils. The instrument is also more easily moved about.

4. *Disturbance Due to Magneto-striction.* The simplest way to mount the parts that carry the string is to fasten them directly to the top and bottom of the polepieces. However, if the polepieces are massive, the magnetostrictive change of form may produce a slight tilting of the string mounting when the field is excited thus impressing upon the string a small angular component of tension. If the string is under considerable tension, the slight angular component thus introduced may produce no visible effect. If the tension is reduced to a value

comparable in magnitude with this component, the zero or focal plane or both will be altered on exciting the field.[6]

C. THE MICROSCOPES

An outstanding feature of the Einthoven galvanometer is that no dead load is carried by the moving part for the purpose of magnifying the motion. For this a microscope is employed. The optical system adopted for the original instrument is a Zeiss 4 mm apochromatic objective, of numerical aperture 0.95 as projection lens[7] in conjunction with a suitable projection ocular, such as Zeiss projection ocular No. 2 or No. 4. To illuminate the field a Zeiss 4 mm achromatic objective of N.A. 0.85 is used as condenser. For special purposes it is sometimes advantageous to use as condenser an apochromatic lens similar to the projection objective. These microscopes are mounted in borings in the polepieces and provided with focussing fine adjustments. In the Leyden instrument each microscope was provided with a double cross slide for centering. If one is to realize the full advantage of an optical system of the quality above mentioned, adequate means for centering must be provided.

The design of the mounting of the microscopes is a matter requiring careful consideration. The usefulness of a sensitive measuring instru-

[6] The writer first observed this effect on a large instrument designed for the Rockefeller Institute for Medical Research. The effect was at first attributed to molar motion of the polepieces, although care had been taken in design and construction to insure against this. After it had been shown that the effect observed could not be so accounted for, its real nature was suspected and the suspicion confirmed by experiment. The question then arose why this effect had not been observed in the Leyden Model. In this model there is a large bronze plate through which the cores of the magnet pass and to which the polepieces are fastened. The support for the string mounting is fastened to the middle of this bronze plate where, presumably, the magnetostrictive change in the polepieces produces the least movement. In the Rockefeller instrument the effect was noticed when the tension on the string corresponded to a sensitivity of about 10^{-7} ampere/cm. It disappeared entirely when the tension was increased fourfold. In the Columbia copy of the Leyden instrument no effect of this kind was observed until the tension was reduced to an amount corresponding to a sensitivity of 10^{-6} ampere/cm. At this sensitivity the effect was well marked. The Rockefeller instrument was made to operate satisfactorily by bolting a bronze plate to the back of the polepiece with shelf-like projections above and below, but not touching them. The string housings were then mounted on these shelves. In subsequent constructions of the same type in the Rockefeller instrument the string housings were carried on a bronze girder mounted on the polepieces with a Kelvin point-groove-plane arrangement which permits the greatest possible opportunity for change in the polepieces without strain of the string supports. This mounting will be illustrated in connection with the description of the instrument of which it forms a part. See Section 2, Article 2, of this paper.

[7] In correcting for spherical aberration the lens maker usually allows for the use of a coverglass. Lenses for use with a string galvanometer should be mounted [illegible] for use without coverglass.

ment is greatly enhanced if it can be used in situations where there is much mechanical vibration without special precaution. A moment's reflection will show that the string itself will be but little influenced by mechanical vibration so long as it is under tension. Its inertia is so small that it will follow any movement of the frame of the instrument without appreciable lag. On the contrary the microscopes have a great deal of inertia and every joint in their mounting affords possibility for movement. Even solid metal is far from rigid. The microscope mountings should be designed for lightness with the maximum amount of rigidity and the minimum number of joints consistent with providing the necessary adjustments. Such joints as are unavoidable should be made with the utmost care. When the galvanometer is subjected to vibration there is a tendency for the microscopes to lag behind the movement of the frame and as the string does not lag, the relative movement is magnified to the same extent that the microscope magnifies the movement of the string in making measurements. If the galvanometer as a whole moved one tenth of a millimeter the movement would be just detectable on a photographic record. If, as is often the case, the magnification is 1000 diameters, a relative movement between string and microscope very much less than this is beyond the limit of tolerance.

When the string is mounted in a protecting case it is usually not practicable to use lenses of as high aperture as the 4 mm apochromatic on account of the very short working distance (about 0.2 mm) of this objective. Use of lenses of lower aperture imposes limitations as to magnification and possible speed of photographic registration which should receive careful consideration in the choice of a design. If extreme sensitivity or registration at high speed is required, the construction must be such as to permit the use of lenses of the highest available aperture. Achromatic lenses are satisfactory for subjective observation and for photographic registration at moderate speeds and magnifications. The Spencer Lens Company has recently made some special achromatic lenses for string galvanometer use with a view to good photographic definition rather than to excellence of the subjective image. These are less expensive than the apochromatics and give very good results. In some work optical filters may be used with advantage to improve definition.

D. STRINGS

1. *Quartz Strings, Chemically Silvered.* In some of his earlier experiments Einthoven tried strips cut from aluminum leaf (*1*, p. 1064). He

presently resorted to fibers of quartz glass (fused silica), coated with silver to make them conductors of electricity. The silver coatings were at first produced by one of the chemical reduction methods such as are used in silvering glass mirrors. These coatings are rough and tend to become more rough if the deposition is allowed to go on too long in one continuous reaction. The process may be controlled so as to diminish the roughness by silvering in two or more stages and by regulation of the concentration and temperature of the bath. The fibers may be polished by stroking them with a fine copper wire. Einthoven made excellent strings in this way and the process was described by Salomonson (8). The writer made many strings in this way and it is less difficult than might be imagined. A piece of copper wire about No. 40, B&S gauge, and 10 cm long is suitable. This should be fixed in a light handle such as a quill. One causes the wire to vibrate by slight motion of the wrist and under a shaded electric lamp brings the string stretched on a frame up to the vibrating wire in such a manner that the direction of its length is in the plane of vibration of the wire. The dark space under a table makes a good background against which to see the illuminated string. Strings of diameter as small as .0015 mm will withstand a surprising amount of lateral pressure, but care must be exercised at the beginning of the process that the wire does not catch on some of the larger roughnesses as the string is then easily broken. The polishing process is facilitated by securing a deposit of good quality, but it may take considerable labor to produce a really smooth and satisfactory string. Often the conductivity is lost after the first polishing and the silvering must be repeated. The coating should be examined from time to time with a lens to observe the progress of the work. Because of the probability that the silvering will have to be repeated it is most essential that the wire used for polishing be perfectly clean and never touched with the fingers.[8] Otherwise the string may be contaminated and new silver will not deposit firmly on a spot soiled with grease, etc. Fibers to be coated chemically should be silvered as soon as made and polished and re-silvered if necessary at once. If blank fibers are kept, even under cover, they quickly collect dirt and seem to undergo some surface change which makes it impossible to secure durable coatings. If fibers

[8] It is an excellent plan to brighten the wire by stroking it lengthwise with fine sandpaper and then electroplate it with silver in a bath of potassium-silver cyanide. After plating rinse the wire in distilled water and do not allow it to come in contact with anything except the string thereafter. It should be noted that in the silvering process the utmost cleanliness must be maintained and details followed with meticulous (almost ceremonial) care.

are not polished the mass for a given conductivity will be greater than that of a smooth string. The shadow of a smooth string photographs more sharply than that of a rough one.[9] The conductivity of a chemically silvered string usually increases for a time due to condensation of the deposit and then diminishes. Failure to wash out all traces of chemicals hastens deterioration. The polished strings usually have a longer life, perhaps because loose metal is rubbed away and what remains is more adherent.

2. *Quartz Strings Coated by Cathodic Sputtering.* Much better strings can be made by cathode disintegration in vacuum (9), (10). Silver may be used in this process also and is the metal originally used by the writer. While it possesses certain theoretical advantages on account of its high conductivity and low specific gravity, the absolute amount of metal required is usually so small that gold may be substitutd withs advantage. When gold is used the excellence of the coatinges is les likely to be impaired by slight accidental contamination of the vacuum with various vapors which are very prejudicial to silver. Strings made by this process last for years if properly handled and will even stand considerable abuse. One silver coated string was in use for five years in the Columbia laboratory. It was finally laid aside wile still serviceable because a string of different properties was requirhed.

3. *Strings Silvered by Evaporation of Metal at High Temperature.* The writer has also coated strings with silver by evaporation of the metal at high temperature (but below the melting point), in a very high vacuum. The process has the advantage that coatings of suitable resistance can be secured in from two to four *minutes* instead of the two or three hours required by the methods of cathode sputtering. However, the difficulty of producing and maintaining the necessary high vacuum sufficiently free from vapors which may react with the vaporized silver is very great. The difficulty is still greater if one employs an apparatus with ground joint which can be used repeatedly. Gold could not be used as it does not evaporate below its melting point. It seemed easier to develop a method for coating large numbers of fibers by cathode sputtering at one time and the method of rapid evaporation by heat was not further elaborated.

4. *Properties of Quartz Strings.* It may be of service to point out some properties of strings which are not generally known. In making the

[9] It is customary to use a cylindrical lens in front of the slit of the photographic recorder. If the string is rough, the reduced image of the shadow formed by the cylindrical lens will not have well defined edges.

blank fibers[10] it sometimes happens that a fiber will be observed to be distinctly curly. Such a fiber should always be rejected. It may be caught and stretched so that it looks well and it may be coated and used successfully provided it is always used under tension sufficient to keep it straight. If one tries to use it at such a tension that the tendency to curl can manifest itself, the zero and focal plane will be found to change with every alteration of tension. It may also go out of focus as it is deflected across the field. These are very annoying characteristics and limit the usefulness of the string seriously. Fibers may also be tapering, elliptical in section or ribbon-like. All such as well as curly fibers should be rejected. The value represented by a blank fiber is much less than that of the labor involved in coating, soldering and mounting in the galvanometer and incomparably less than that which the user will waste in trying to make measurements with it if he ever uses it slack.

Strings may acquire paramagnetic properties by contamination with iron dust. For this reason every care should be exercised to keep them protected from dust while in storage. After mounting in the galvanometer they should not be unnecessarily exposed. They seldom become magnetic while in the galvanometer, but the writer has seen this happen as a result of rubbing away a small rust spot on one of the polepieces while the string was exposed.

An otherwise good string may have its value much impaired in soldering it to the brass tips if through careless or unskillful manipulation it is given a torsion or is embedded in the solder in a direction which makes an angle with the direction the string will take when stretched. Einthoven pointed this out (7, p. 315), and indicated how the difficulty might be overcome by use of certain adjustments. The Leyden galvanometer and the one described in Section II, §2, as Type A are provided with all these adjustments. Type B, (Section II, §3) has one of these adjustments, but it is not altogether easy for the average user to make use of them. If care is used in making the strings it will seldom be necessary to attempt these unusual adjustments except in connection with very critical work at small tensions.

A good quartz string free from the above mentioned defects and centered properly in the field of a well made galvanometer can be slackened or tightened at will without showing change of zero or focal plane so long as it is under moderate tension. Even when the tension

[10] For the method of making blank fibers see Appendix G.

has been greatly reduced the change in zero and focal plane should be very slight and after re-focussing a very slack string it should be possible to deflect it entirely across the illuminated field without material change of focus and it should return to a practically constant zero, which, however, may not be quite the same as the zero under greater tension. Under any given tension the zero will remain constant within about one tenth mm, this figure referring to the shadow magnified 1000 diameters. To one accustomed to work with delicate torsional instruments this constancy of zero is one of the most striking features of the Einthoven galvanometer.

It may not be out of place to explain why so much stress has been laid on this matter of alteration of tension without change of focus and zero. The string galvanometer is used almost entirely as a deflection instrument and largely with apparatus for photographic registration. Its sensitivity can be altered and adjusted with great precision to any predetermined value in a small fraction of a minute provided the requirements above outlined are met. This is largely a matter of design and of care in providing good strings and as it will facilitate all work to be done with the instrument it is distinctly worth while. No one who has ever worked with a galvanometer possessing these good qualities will ever care to work with one that lacks them.

5. *Metal Strings.* Fine metallic wires made by the Wollaston process (11) may be used as strings. They have been largely employed in continental Europe. Those most generally used have been of platinum. No metallic wire yet made approaches the excellence of a good coated quartz string for most purposes. Before smooth strings coated by cathode sputtering were available some users of the string galvanometer preferred platinum strings because of their smoothness and the clear shadow they cast on the photographic surface when used with a recorder provided with a cylindrical lens. This distinction they no longer enjoy exclusively as the fibers coated in vacuum are quite as smooth as the best Wollaston wires. Platinum has a high specific gravity and a comparatively high specific resistivity. Its tensile strength is low. In order that the mass of a platinum wire shall be small enough to make it suitable for use as a galvanometer string it must be of very small diameter and it is then very frail. Platinum strings, at least those made from Wollaston wire commercially available, are always distinctly paramagnetic and as they are usually slightly curly and used at small tension they invariably in my experience change zero and focal plane on exciting the field in spite of the most careful centering. Notwith-

standing these drawbacks they have been much used and good work has been done with them.

Aluminum wires made by the Wollaston process were first used as galvanometer strings by the late Professor J. K. A. Wertheim-Salomonson of Amsterdam (12). Aluminum has a low specific gravity, is a good conductor of electricity and its magnetic susceptibility is about half that of platinum. It would be an ideal material for strings were it not for its low tensile strength and the fact tht it seems to have no particular elastic limit, but "flows" under tension. Strings of this metal will not last for any great length of time unless used at very moderate tensions. It is difficult to make them of very small diameter, but because of the low specific gravity of aluminum this would not be a serious objection and for some work might be an advantage. The really serious difficulty about their use is the yielding under tension. They may prove useful in some of the physical applications of the galvanometer where very short period is not necessary and low resistance is desired.

Gold wires are easily prepared by the Wollaston process. The susceptibility of gold (diamagnetic) is still smaller than that of aluminum, its specific resistivity is not great and its tensile strength is greater than that of aluminum, though less than that of platinum. It is less easily drawn into extremely fine wires than platinum and would be best used for work requiring low internal resistance and not very short period. Its specific gravity is high. Wires of copper and silver of such dimensions as can be drawn directly without resort to the Wollaston process may sometimes be serviceable as strings for special purposes such as use of the string galvanometer as a vibration galvanometer, tuned to the frequency of the impressed current. The alloy duralumin is light and has a tensile strength much greater than that of aluminum. I have never been able to get wires of this alloy of very small size. It is very stiff, but may be useful for special applications. Ductile tungsten is drawn commercially to a diameter of .01 mm. It has a high tensile strength, but is very stiff and has a high specific gravity. It was used as string material in the sound ranging galvanometers during the war. It curls badly and cannot be used except under tension sufficient to straighten out the kinks.

The metal glucinum, otherwise known as beryllium, is a ductile element of specific gravity 1.9. It is a good conductor of electricity. If metallurgists can produce this material in the form of fine wire it might be very useful as string material for some purposes, though I have no information as to its tensile strength or elasticity.

6. *Handling of Strings.* On account of their extreme tenuity quartz strings and fine metallic strings are not handled directly, but are stretched on small frames, usually of glass, on which they are coated (or corroded in the case of Wollaston wire).

From these frames they are transferred to brass tips by placing the tips in a suitable clamp which is held in a vise, while the string on its frame clamped in an adjustable stand is moved into position so that it comes into contact with the tips to which it is then soldered while still stretched on the frame. A string when stretched is easily visible to the naked eye when brightly illuminated. This is true even of strings so thin as not to be resolvable with a low aperture microscope. With practice in observing them in light of uniform intensity one can even make a rough estimate of the diameter. This depends on the intensity of the reflected light. If one end of a string becomes free while the other is held one gets occasional glimpses, but is not able to see the entire string at once. This is a principal reason for always manipulating them on frames. Wires as large as .01 mm may be handled like larger wires, but even these are more easily handled when stretched.

Strings are stored in holders provided with clamps in which the brass tips are fastened. From these they may be transferred to the galvanometer by means of a special clamp or manipulator which should have a simple tension adjustment. It is also a convenience if the ends of the manipulator are insulated from each other and provided with binding posts so that resistance measurements may be made while the string is in the device. In transferring a string the manipulator is clamped upon the brass tips before these are released from the storage clamps. It is then clamped in the galvanometer without releasing the manipulator clamps which are unscrewed after the tips are firmly fastened in the galvanometer.

7. *Repair of Strings.* Coated quartz strings if accidentally subjected to too great a tension may lose their conductivity. This is usually due to separation of the coating from the solder at the joint. By slackening the string in a manipulator and warming the solder gently with a small copper, it may be drawn further into the solder. In this work it is well to hold the manipulator in a vise and its binding posts may be kept connected with a bridge so that the conductivity may be tested at each step of the operation. If conductivity is not restored by the first attempt, slacken again and draw in the other end. This will usually be effective and the final resistance should not differ much from the value before the accident. These fibers are usually soldered with the alloy

known as Wood's metal which melts at 70°C. The copper used in soldering should be heated over a flame until the water of condensation evaporates again. It will then be hot enough to melt the alloy and if of small size it will cool rapidly which will facilitate its withdrawal, leaving the string held by the solidifying metal. The useful life of a string may often be greatly extended by repair in this manner.

Section 2. Description of Instruments

1. *The Leyden Model.* The Leyden Model is fully described in Einthoven's paper (7) on construction which may be consulted for additional details. The principal features are an electromagnet consisting of two iron cores 8 cm in diameter and 31 cm long, connected by a heavy yoke of rectangular section 29.5 cm long, 14.8 cm high and 6 cm thick. At the end opposite the yoke the cores pass through holes in a heavy bronze plate to which the polepieces are fastened. The polepieces, also of rectangular section, are 12.5 cm high, 6 cm wide and 8 cm long exclusive of the wedgeshaped ends. The top angle of the wedge is 90° and the polefaces are 2 mm wide. The airgap is 2 mm long measured in the direction of the lines of force. The polepieces are bolted to the cores as well as to the bronze plate and the wedgeshaped spaces between them are filled with snugly fitted brass wedges. The rear wedge is held in place by the bronze plate and that in front by two heavy bronze bars. The polepieces are thus firmly braced against mechanical stress produced by the magnetic field. The cores are wound with 1848 turns of copper wire 2.2 mm in diameter, the resistance of the coils in series being 2.6 ohms. Originally this instrument was provided with leveling screws, but these were abandoned as it was found that notwithstanding the fact they were well fitted the slight play magnified the effect of mechanical vibration ten fold. Other features of this instrument have been mentioned already. In point of convenience of operation, precision and sensitivity this original instrument left little to be desired. It has, however, one disadvantage: it is essentially difficult and expensive to construct.

2. *New Design, Type A.* In 1916 the writer described at a meeting of the American Physical Society in New York City, a new construction of the string galvanometer.[11] The accompanying figures, 1, 2 and 3, are

[11] The design of this instrument was undertaken at the request of the Rockefeller Institute for Medical Research when the coming of the war in 1914 prevented them from procuring the contruction in Europe of a copy of the Leyden Model. The modifications were made to reduce the expense of construction. The form of the magnet was suggested by that of the older half-string magnet of DuBois (13).

from photographs of this instrument, which for brevity will hereafter be referred to as Type A. In the Leyden Model soft Swedish iron was

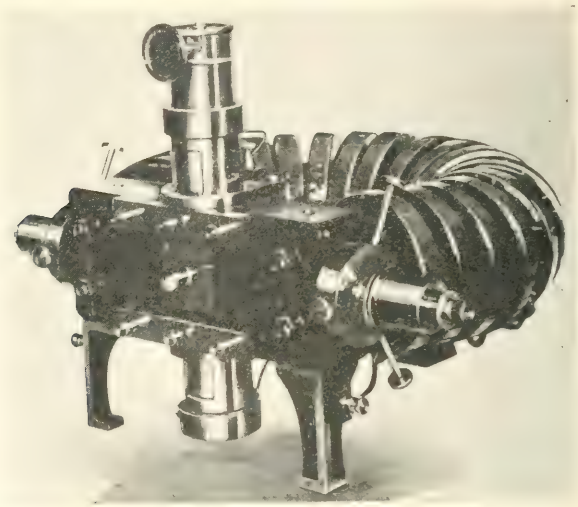

FIG. 1. *Type A. All parts in place.*

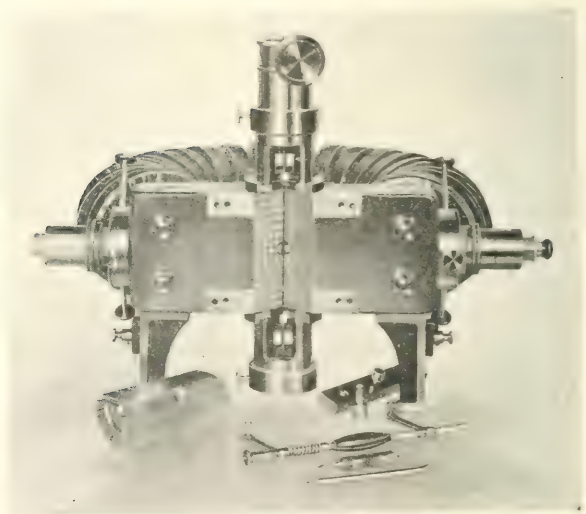

FIG. 2. *Type A. Front bracing bars and wedge removed. String housings open. Device used to insert strings is shown lying in front of the instrument.*

used for the entire frame of the magnet. In Type A the yoke is a casting of special low carbon steel of high permeability. In the first instruments of this type Swedish iron was used for the pole pieces

When Swedish iron of really high grade is available, it is a slight advantage to use it for pole pieces, but much Swedish iron contains seams of oxide introduced through folding in the forging of the blooms and these seams materially lower the permeability. It is also very tough and difficult to work so that its use adds to the expense of construction. Some instruments made with polepieces of this iron were no better than others made entirely of the soft cast steel. The diameter of the cross-section of the yoke is 8 cm, the same as that of the cores of the Leyden Model. The polepieces are of the same height and width as those of the Leyden Model, but longer to fit the milled-off ends of the

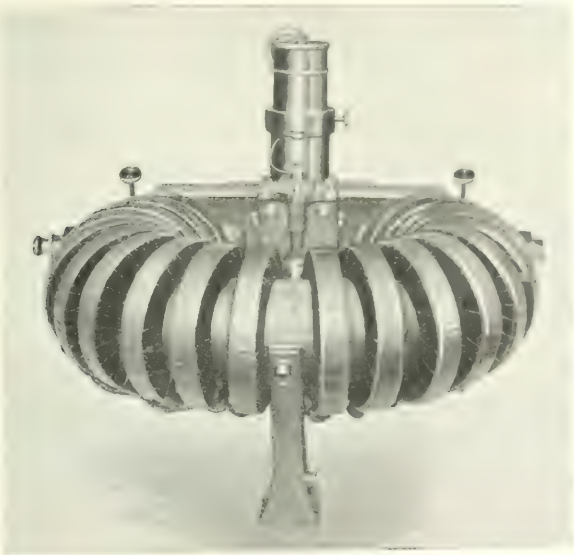

FIG. 3. *Type A. Rear view showing method of mounting string housings.*

ring-shaped yoke. The airgap in most of these instruments has been 2 mm, as in the Leyden Model, but a few have been made with 1.5 mm airgap. These give greater field strength for a given exciting current and offer but little more difficult in the matter of inserting strings, really presenting no difficulty at all to a skilled technician. The exciting coils are of No. 11 B&S gauge copper wire, of which there are 1920 turns. This wire is wound in a series of flat coils. The coils are wound on forms, then taped and connected in series after being strung on the yoke. The resistance of the coils in series is 2.5 ohms.

The strings are mounted in cylindrical housings which can be seen clearly in the figures. The clamps which hold the string tips are visible

in Fig. 2, which shows the instrument with the front wedge removed and the string housings opened. The string tips can also be seen, and lying on the table before the instrument is the manipulator used in removing and inserting strings. The clamps which hold the string tips are adjustable parallel to and perpendicular to the polefaces, by screws, two of which show in the figure. The upper housing is taller and contains in its upper part the adjustment for altering the tension of the string. This adjustment consists of a screw driven by a worm and gear and opposed by a spring. The airgap is closed in by brass wedges as in the Leyden Model. In Fig. 2 one of the wedges is lying on the table and in Fig. 1 it is shown in place. These wedges are scraped and ground to a close fit. Cylindrical gates which rotate in the housings complete the closure, the arrangement being evident in the figures. Besides the opening in the front of the housings there is one at the side also closed by the rotation of the gate. This admits the screwdriver when a new string is to be clamped in place and also permits the operator to view the string laterally so as to judge of the centering in the antero-posterior direction. In observing the lower end a small mirror is used for the lateral view. When the wedges are in place and the gates closed the string is well protected against dust and air currents, so that it is not necessary to cover the entire instrument.

The polepieces are braced in rear by a small bronze plate which fits between the butt ends of the iron ring and is screwed to the polepieces. In front are two bronze bars which further brace the instrument and hold the front wedge in place. Both string housings are mounted on a massive bronze carrier, the back of which can be seen in the rear view, Fig. 3. This carrier is held against the rear bronze place by heavy bronze spiral springs and is located by adjustable screws whose tips rest in a point, groove and plane respectively, the well known arrangement of Lord Kelvin. The upper and lower plates of this carrier do not come in contact with the top and bottom of the polepieces, but have a slight clearance. Felt washers let into these plates make a dust-proof joint and prevent disturbance by air currents.[12]

The microscope tubes slide in sleeves bored and lapped individually to as close a fit as will permit the necessary freedom of movement. The sleeves have a shoulder which fits in a centering box provided with three adjusting screws. The fit of the shoulder in this box is made so close that the friction will hold it in position even if all the adjusting screws are loosened. There are no gibs or springs and when one screw is

[12] In connection with this carrier see footnote 6, Section 1-B, Article 4.

loosened another must be tightened before movement will take place. A centering box so made is incomparably more rigid than the usual constructions and adjustments can be made with it quite as easily. The adjustment once made is far less liable to accidental change than is the case where easy fits and the use of springs and gibs is adopted. No coarse adjustment for focussing is needed. For fine adjustment a cap fits over the microscope and against a shoulder on the tube, a ball bearing thrust collar being interposed. The tube is pushed out by a coiled spring made large enough to slip freely over the tube. The cap screws to a threaded collar on the sleeve. The screw threads of this adjustment are carefully made on a thread-milling machine and in spite of the large diameter they function smoothly. Adjustable screw stops are provided so that the microscopes can never be screwed in far enough to touch the string. They require to be adjustable as the lens mountings vary somewhat in length. When once adjusted no further change is needed unless the lenses are changed. As the mountings of objectives vary in length, care should be taken to see whether the stops require readjustment if lenses are changed.

In the Leyden Model there was an opening from the airgap along the microscope tubes which required to be packed lightly with soft paper to prevent disturbance of the string by air currents. This opening is effectually closed in Type A by the manner of mounting the microscopes.

The entire instrument stands on three legs without leveling screws. The instrument has been used to record the human electrocardiogram in a factory building where a large number of machine tools were in operation, without any disturbance from mechanical vibration appearing in the photographic records. The instrument stood on an ordinarily substantial wooden table, but no other precaution against disturbance was taken except the constructional features of the instrument itself already mentioned. Besides the rigidity of the microscopes the power of the optical system and the strength of the magnetic field contribute to the result as the string is tighter for a given sensitivity than it would be in a less powerful instrument. Special emphasis should be laid on this comment as the shortcomings of inferior designs have led to a widespread opinion that the string galvanometer is especially susceptible to disturbance by vibration and requires elaborate precautions to protect it. The reverse is true of a good instrument and *there is probably no instrument comparable in current sensitivity with the string galvanometer which is so little affected by vibration.* Type A was planned

for laboratory use, but on account of the difficulty of obtaining satisfactory instruments for clinical use during and immediately following the war years a number of these large galvanometers were supplied to hospitals and clinics. In the spring of 1919 the maker[13] suggested that the design of a new model be undertaken with a view to providing an instrument suited particularly to the needs of physicians and capable of being manufactured at a lower cost than Type A.

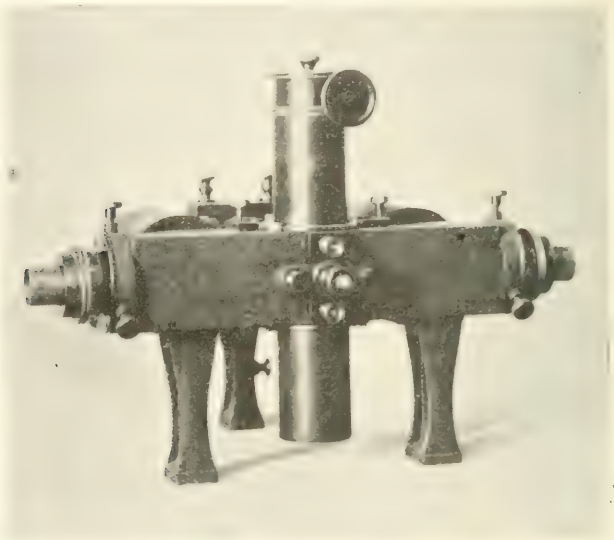

FIG. 4. *Type B. All parts in place.*

3. *New Design, Type B.* The problem of designing this new instrument had not been long under consideration when it was realized that it would be possible to make it not only a smaller and more convenient instrument for clinical use, but also a precise galvanometer of comparatively small size which would be capable of doing most of the work for which the large galvanometer is suited and even better adapted for some applications. Figs. 4 and 5 show this instrument, the design of which was completed in the spring of 1920. The result desired in Type B was an instrument of smaller weight and less cost, but of quality equal

[13] These instruments were made by Mr. Charles F. Hindle. I take this opportunity to acknowledge my indebtedness to his mechanical skill and to express my appreciation of his conscientious efforts to meet every requirement. Though the writer is responsible for the essential features of the design of these instruments, even to many of the minor details, much was left to the discretion of Mr. Hindle and he has initiated various improvements. The manufacture is now carried on by the Cambridge Instrument Co., Inc.

to that of Type A. It was desired to retain the same optical system and at the same time make such arrangements as to minimize the special skill required of the operator.

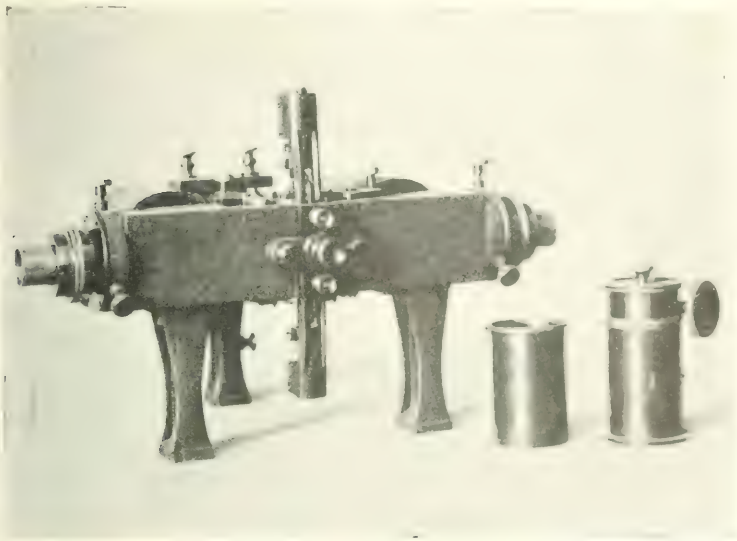

FIG. 5. *Type B. Upper and lower string caps removed, string carrier in place.*

The size of the magnet is determined by the dimensions of the airgap and the field strength desired. The height of the airgap depends on the string length. If strings were perfectly flexible and if it were not necessary to perforate the magnet for the optical system, the obvious

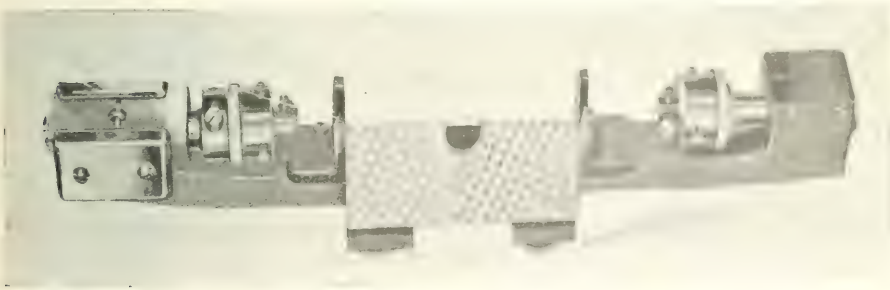

FIG. 6. *String carrier for Type B.*

way to make a small galvanometer would be to reduce the length of the string indefinitely. Previous experience with a galvanometer hastily constructed in the writer's laboratory some eight years before to meet

a special requirement had provided the information that a pole length of six cm was not too short for good results and this length was chosen for Type B. Another factor which affects the size of the magnet is the leakage flux. With high flux density in the airgap this is certain to be large and with a distributed winding it will be somewhat larger than with a concentrated one. The principal reluctance to be overcome is in the airgap and the shorter we make this the less will be the coefficient of leakage and the smaller the number of exciting turns may be. With a short gap the diameter of the iron cores may also be reduced.[14]

Apart from the reduction in height of the polepieces, the chief feature in the design of Type B which makes possible reduction of size and weight is the length of the airgap which has been made one half mm. The construction of the string mounting is shown in Fig. 6. It is made in a single removable unit containing the centering devices and also the spring and slide of the tension adjustment. To center the strings this mounting is put into a special fixture made of hardened cast steel, carefully seasoned and ground to dimensions with great precision. In this fixture the string can be centered with the necessary accuracy, the work being controlled with a low power microscope provided with an ocular micrometer. Guides are provided on the mounting which fit corresponding parts on the magnet so that the centered string can be inserted without danger of coming into contact with the polepieces or the lenses. The microscopes are centered on the string and with each other. By the use of suitable jigs, fixtures and gauges in manufacture it is possible to make the magnets so nearly alike that when the microscopes have once been centered on any string which has been located in the mounting by means of the centering fixture, any other string centered in this fixture may be inserted in the galvanometer and its shadow will at once appear at or near the center of the illuminated field. This is an advantage to the user who does not wish to incur the risk and trouble of handling strings and centering them himself. It is also convenient if one wishes to keep at hand several mountings containing strings of different resistance, etc. These may

[14] It was part of the original plan of this paper to give in some detail the calculations on which the design of this magnet was based. The paper has grown to such size that it seems best not to expand it unnecessarily and it appears probable that anyone who would be likely to make use of magnetic data in design would already be fairly familiar with the subject. An excellent practical treatment is to be found in "The Magnetic Circuit," by V. Karapetoff, McGraw-Hill Book Co., New York, 1911. DuBois' "Magnetische Kreise" will provide additional data and bibliography.

be interchanged in a fraction of a minute. When not in the galvanometer the mounting and string can be kept in a dustproof metal box in which it may also be shipped conveniently. The construction is such that the skilled technician may insert and center his own strings while those who prefer may have this service performed for them by the maker. When the mounting is in place the lower end is covered by a cap provided with a bayonet joint. The upper end is covered with a housing which carries the wormgear and screw of the tension adjustment. The microscopes are similar in all respects to those used in Type A, except that it has been possible to reduce the tube length to 160 mm. In Type A the tube length is 250 mm so that objectives corrected for use in the one type should not be used in the other.

The magnet frame consists of two similar castings of high permeability steel, cores and polepieces being cast in one piece. There is no separate yoke, but the cores curve around behind and terminate in flanges which bolt together in the midline opposite the airgap. There is thus but one joint in the iron. The magnet coils are disposed similarly to those in the Leyden Model, but the legs are separated further. There are 1000 turns in the exciting coils equally divided between the two legs and their resistance in series is 1.5 ohms.

4. *Other Models.* It would require much space and some information not at the writer's command to describe all of the various constructions which have been made either privately or commercially. We shall mention a few which are important either from the large number in use or because of their interest in connection with the development of this instrument. Dr. Einthoven has developed several types since the construction of his original model[15] and these he will probably describe soon. One very interesting model has been described recently by his son, W. F. Einthoven (14). In this instrument the strings are extremely short and are mounted in the space between the lenses so that the field available for operation is merely the leakage into the region of the perforation. It is, nevertheless, sufficient.

The late Dr. J. K. A. Wertheim-Salomonson designed and built privately several string galvanometers. In these instruments the windings were concentrated and the iron so arranged as to give two parallel magnetic circuits. The string is mounted on a removable member. Another model with concentrated windings was designed by Dr. George Fahr for Professor L. Asher, of Berne. An instrument based on this

[15] Oral communication.

design, but considerably modified, is built commercially by Stoppani of Berne. Boulitte, of Paris, builds a simple and compact string galvanometer designed by Mr. Lucien Bull, of the Institut Marey. This instrument is developed from the small instrument designed by Mr. Bull during the War as a part of the Bull-Tucker sound-ranging equipment. I have not seen this instrument and know it only from the catalog of the maker. It seems to lack certain adjustments which I regard as essential in an instrument adapted to general service, for example I can see no provision for the accurate centering of the strings. Presumably it has been found serviceable for the work it is designed to do, clinical electrocardiography.

The Cambridge Instrument Company, Ltd., of England, has made a large number of string galvanometers after a design by the late Mr. W. Duddell. In this instrument the string is enclosed in a thin brass box provided with thin mica windows at the center. The box hangs on a point-groove-plane support which permits it to be adjusted in the airgap and there is a screw adjustment in a direction at right angles to the direction of the lines of force. The enclosure of the string offers obvious advantages, but as the lenses cannot be brought close to the string, their possible aperture is limited. The usual equipment is a 16 mm apochromatic objective of N.A. 0.30 as projection lens. It is possible to use an 8 mm objective of N.A. 0.65 by bringing the string close to one side of the box, but this can be done only if the string is to be used under tension sufficient to keep it taut. The advantage apart from better optical definition is that at the higher magnification thus made possible the range of proportionality of the instrument is increased. This instrument is also supplied with a box, or "vibrator case," containing two strings which may be projected simultaneously on the same photographic registering device. This is useful for recording two related trains of events, such as current and voltage. In the original design there was a single large exciting coil placed on the yoke opposite the airgap. This was not an ideal design from the standpoint of energy consumption and avoidance of leakage and the instrument is now made with the winding distributed in two coils on the legs of the magnet. This instrument is satisfactory for electrocardiography, for which it has been largely used, and for other applications where the string is under tension. It can be used with the string slack, but is less convenient in this condition than an instrument provided with screw adjustments for centering the string, as the centering by swinging the box is not so easily accomplished. On account of the method used for adjustment of the string in the box there is apt to be lateral and torsional

stress at the ends of the string which causes more change of zero and focal plane than occurs with the Leyden type or instruments based on it. The change of zero is readily compensated by the lateral adjustment screw and if the 16 mm lens is used there is sufficient working distance to permit of keeping the string in focus. The microscope mounting is a geometrical clamp which makes this instrument also very insensitive to vibration.

Edelmann of Munich was probably the first instrument maker to build string galvanometers commercially. His first three instruments were based on the Leyden Model and were very similar to it. He then designed an instrument with concentrated windings whose form was evidently suggested by the large Weiss electromagnet. A considerable number of these instruments came into use between 1907 and 1914. The string is mounted on a separate carrier with centering screws and a pinion and gear tension adjustment. The optical system was that adopted by Einthoven. The instrument is nicely made, but certain features of the design are not too happily chosen. Briefly the principal criticism pertains to lack of rigidity in the magnet frame and in the microscope mountings. The string carrier is also lacking in solidity. The magnet frame deforms on exciting the field, so as to change the alignment of the microscopes and destroy the parallelism of the pole-faces. This difficulty can be overcome by bolting across the top of the frame a rectangle of cast iron with one side removable to permit insertion and removal of the string carrier. The instrument was evidently designed with a view to exclusion of dust and air currents, but the means adopted are insufficient and the instrument should be covered with a box. It is decidedly sensitive to vibration on account of the manner of mounting of the microscopes. Removal of the leveling screws is a help and if necessary the entire instrument may be mounted on a Julius suspension. On account of its excellent optical system it is worth while to make these alterations. The writer has used an Edelmann galvanometer so altered with good results. Edelmann also constructed a large model with permanent magnets and several smaller models, some with permanent magnets and some with electromagnets. The latter heated badly and would not maintain constant sensitivity long enough to be used for serious measurements (15). The permanent magnet small type was lacking in sensitivity and had no considerable range of proportionality. The adjustments on these small instruments were extremely crude. There was also an Edelmann instrument with two strings in one magnetic field which I have not seen.

Another galvanometer designed for clinical use is by Huth of Berlin. This has a ring-shaped frame with polepieces projecting inward so as to give two parallel magnetic circuits. The windings are concentrated over the poles. The first instruments must have heated from the energy dissipated in the magnet coils as later instruments were equipped with metallic fin radiators embedded in the windings and projecting out for a considerable distance. The string mounting is detachable and provided with a cover which is automatically removed as the mounting is screwed into place. There is thus combined the advantage of the protecting box with the advantage of using the naked string so that lenses of high aperture can be employed. I do not know what lenses were provided. The instrument I saw did not give good optical results, perhaps because the lenses could not be centered. I believe this was supposed to be done once for all by the maker.

During the war an instrument designed by Mr. Lucien Bull was much used for sound ranging. Six strings were mounted in one magnetic field. These instruments were not used as current measurers, but as indicators of the time of beginning of a current. It was unnecessary to provide elaborate adjustments either for centering or for regulation of tension. The strings were mounted on a frame provided with insulating bridges and spacing pins and were fastened at one end to fixed pins and at the other end to pins which could be rotated like the keys of a banjo. The tension could thus be regulated to a suitable value and as the variations due to temperature change made no material difference the fact that the adjustment was not particularly convenient was not objectionable. This string mounting from its peculiar construction was aptly known in the Service as a "harp." These instruments were extensively and effectively used in the English, French and United States armies. The sensitivity did not require to be great and low power optical systems were used, the projection lens being a photographic objective giving a magnification of about thirty diameters. The six strings spaced about a half mm apart made a system whose magnified shadows were a considerable distance apart. Each shadow was intercepted by a small prism which picked up a small part of the adjacent pencil of light. By suitable adjustment of the six prisms, the six small pencils of light could be brought side by side on a strip of photographic paper some three cm wide and so aligned that simultaneous movements of the wires would fall on the same ordinate of the record. Such an arrangement has obvious physical and engineering applications.

Sanborn, instrument maker of Boston, has recently placed on the market a string galvanometer of small size as part of an equipment for clinical electrocardiography. The writer has seen one of these instruments, but has no data as to its constants or performance.

SECTION 3. DETERMINATION OF THE PHYSICAL CONSTANTS

1. *The Strength of the Magnetic Field.* The strength of the magnetic field may be determined in several ways. Einthoven [7] has employed a balance method as has Wertheim-Salomonson [6], who used also the method involving change in resistance of bismuth wire. The use of an exploring coil and ballistic galvanometer is an obvious method and may be applied in several ways. A variation of this last method would be the employment of a direct reading fluxmeter, but on account of the smallness of the airgap a coil of many turns cannot be employed and the ballistic galvanometer will give better results.

The curve of magnetization may be obtained from a number of observations by any of these methods at different strengths of exciting current. It may also be obtained as was done by Einthoven [7] by noting the increase in deflection of a quartz fiber kept at constant tension and carrying a constant direct current while the exciting current on the field coils is gradually increased from zero to the maximum value.

Either the maximum or the average field strength can be obtained by any of the above methods. The quantity H, which enters the equations of Part 1, is not the maximum but the *effective* field strength. By this we mean the strength of that uniform field in which the deflection of the middle point of the string produced by steady direct current would be the same as in the actual field. Einthoven has obtained a value of the effective field by measurement of the electromagnetic damping of a quartz string.

Referring to Eq. (6), Part 1, it will be seen that all the quantities are easily measurable except H and m. If m can be determined independently of H, the latter becomes known. It is not possible to determine with any great approach to accuracy the value of m for a coated quartz string from measurements of the diameter and resistance. For the purpose of determination of field strength it is not necessary that the inertia of the string shall be small as all the measurements except that of natural period are made in the state of equilibrium and large inertia in no way hinders determination of the period. If we substitute for the light string a metallic wire of suitable diameter, we may deter-

mine its linear density in advance by weighing a measured length, say a meter. We should, however, bear in mind that Eq. (6) was developed on the assumption that the string is, or is similar to, a perfectly flexible conductor. The quartz string approximates to this assumption so long as the tension is large in comparison with the force required to overcome rigidity. The metallic wire should be put under correspondingly great tension. In any event the rigidity will tend to make the measured value of the effective field smaller than the actual value. To carry out the measurement the wire is deflected by steady current whose strength is measured with a suitable instrument. The deflection of the shadow is measured and the circuit opened. The wire returns to the position of equilibrium after a long series of oscillations and while these are taking place a record of the motion is made on a photographic plate or film moving at the rate of at least a meter a second. Simultaneously there is registered on the plate a record of the time in thousandths of a second. If the tension is suitable, the period can be determined with sufficient accuracy without correction for damping, which, however, is easily made if desired. An independent determination of the magnification is necessary since the quantity l' in the equation is the actual unmagnified motion of the string. Also if the deflecting current is measured in amperes a factor of 10^{-1} must be introduced, since I of the equation is in cgs units.

The possible errors of the several measurements give for the possible error of the calculated value of H a magnitude of one per cent. Considering the use to be made of the result an attempt to reduce this error would probably not be worth the labor involved. This method was devised[16] for an investigation of the Columbia University copy of the Leyden Model and was subsequently used for studying a number of the Type A instruments. The magnetization curves of these instruments were obtained by the method of observing deflections of a quartz string carrying constant current while the field current is varied. For one of these observations the field current is made the same as that used when the effective field is measured by the heavy wire method and thus the values of all points on the curve are determined. The best wire available at the time was some copper wire .048 mm in diameter. The fine tungsten wire now available would perhaps have been better. When this method of measuring the effective field was first applied it was the writer's opinion that there is actually a significant, if not a

[16] This method was described at the February meeting of the American Physical Society at New York City in 1916.

large, difference between the maximum and effective values. This question is perhaps of no great moment in connection with any particular instrument, but is of considerable consequence in the matter of design of new types and will be taken up again in Section IV. The amount of space devoted to description of the heavy wire method of measuring effective field strength is not justified by its importance except for the circumstance that measurements made in this manner have been used as an experimental control to test the validity of theoretical deductions.

2. *The Linear Density of the String.* The linear density, m, may be determined as soon as the field strength, H, and the undamped natural period, τ_0, corresponding to a particular value of the sensitivity, are known. If the motion took place in a vacuum no further comment would be needed. Actually the matter is not simple. In 1828 the astronomer Bessel, in connection with a report of a new determination of the length of the seconds' pendulum, pointed out the necessity of correcting for the inertia of air moved as well as for buoyancy. His experimental results led Stokes (16), (17) to an extended theoretical investigation which included consideration of the motion of a cylinder in a viscous fluid. Stokes arrives at the conclusion that the inertia and also the coefficient of mechanical resistance, k, will be enormously increased by the moved air when the radius of the moving cylinder becomes small. The theory predicted a very large effect for cylinders of small radius, but Stokes was inclined to doubt the accuracy of the predictions when the radius becomes very small. He thought the damping would be even greater than the theory would predict (17), p. 67. It appears from Stokes' work that the additional inertia will be greater the slower the motion.

In working out a method for correction of curves when the galvanometer is used overdamped, Einthoven (18) obtained results which led him to conclude that when the string moves slowly it behaves as if its mass were increased. It appeared to have different values of m during various stages of a single deflection. He also found this effect greater for a string of smaller diameter. The effects he observed were large, amounting in some instances to a six fold increase of m. At the time of publication of these results (1906), Einthoven was probably not familiar with Stokes' work, as he does not mention it and speaks of the result as quite unforeseen. The interpretation he placed on certain of his observations led him to regard k, the coefficient of mechanical resistance, as constant over a wide range of tensions and

in making the measurements from which the change of mass was inferred he determined k for a single point of the curve where it could be measured with greatest precision and assumed that this value held for all other points. Now Stokes' theory, as already mentioned, points to an increase of k and m *both* as the motion becomes slower. One striking experiment described by Einthoven is the following:[17] Two curves were registered showing the response of the string to sudden application of steady current. In the second record the tension on the string and the speed of the photographic plate were both increased ten fold. The current through the string was similarly increased so that the deflection in both records was the same. The shapes of the curves were almost identical. But this experiment really proves that the *damping*, $k/2m$, is constant, and in connection with Einthoven's other observations affords experimental grounds for belief that Stokes' theory is at least qualitatively applicable to cylinders of very small radius. Both these curves were registered at sensitivities greater than that at which variation of m was observed, so that *a fortiori* there must have been alteration of m between these two records. That being admitted, we must conclude that k also changed and the remarkable deduction to be made from these observations is that *the ratio between k and m remains constant.* Stokes' theory points to the conclusion that for a certain range of values of the radius of the cylinder such a compensatory variation of these two quantities might be expected. Stokes' paper is long and the mathematical analysis is difficult. It would require a very extensive digression to consider it in sufficient detail to permit introduction of numerical computations based on it for comparison with the observations on the string galvanometer. The writer hopes to have an opportunity to accumulate experimental data shortly for the express purpose of comparison with Stokes' theory and the results will be communicated later. As experiments of the writer, discussed in Appendix C, have shown constancy of the ratio k/m over a range of tensions between that corresponding to critical damping and a tension somewhat below the maximum safe limit, it may be concluded that the constancy of this ratio holds over the entire range of sensitivity to which the differential equations of motion apply and probably beyond that range since in Einthoven's experiment the lesser tension corresponded to a sensitivity of 10^{-9} ampere/cm.

The true value of m can be obtained from the data of a steady current deflection at a tension for which τ_0 has been determined. Rearranging

[17] (1), p. 1068 and Figs. 1 and 3, Plate III, and (18), pp. 503–04.

Eq. (6), Part 1, $m = HI\tau_0^2/32L$. It will be shown in the next § that the value of τ_0 can be uniquely determined. Further discussion will be found in Appendix F.

3. *The Free, Undamped Period.* The most direct method of determining this constant is to subject the string to tension sufficient to make its motion distinctly periodic and from a simultaneous photographic record of the motion and of a series of uniform time intervals, determine the damped period and the decrement. From these data the undamped period, τ_0, can readily be obtained.[18] If the string is short or sufficiently massive there is no difficulty in making the determination in this manner. Strings of small mass and of the length usually employed in the larger instruments, say 13.5 cm long and .001 mm in diameter, can hardly be made to oscillate in air enough to permit a determination of any considerable precision without subjecting them to a tension likely to be injurious. In Part 1, pp. 147–48, a method is given for determination of τ_0 when the string is critically damped. Another method applicable to the critically damped string is to impress sine wave alternating current, varying the frequency while the amplitude is kept constant by use of an auxiliary measuring device. The natural period agrees with that frequency of impressed current which gives a deflection half that produced by the steady current of corresponding value. For proof see the curve, p. 143, Part 1. If the source of A.C. contains harmonics, the determination is less sharp. A still rougher approximation may be obtained with great ease from any record of response to sudden application of steady current if damping is critical. If t in Eq. (15a), p. 147, Part 1, is put equal to τ_0, the numerical solution shows that the deflection will be complete to within approximately two per cent of the final value in a time after application of the emf equal to τ_0. If then we measure on a record the elapsed time from the beginning of the motion until it is 98 per cent completed, this will give a rough approximation to the natural period.

In Appendix B it is shown that $\tau_0 = \tau_k \sqrt{1 + \lambda'^2}$, where $\lambda = k\tau_k/4m$. Substituting this value of λ'^2, we have

$$\tau_0 = \frac{\tau_k}{\sqrt{1 + \dfrac{k^2\tau_k^2}{16m^2\pi^2}}}$$

from which it is seen that the value of τ_0 is unique since it depends on the measured value of τ_k and the ratio k/m which has been shown to

[18] See Appendix B.

be constant. τ_0 is thus a true constant. This justifies the statement at the end of the preceding article that the value of m determined from a steady current deflection is unique and is the true value independent of the effect of the air in which the motion occurs.

4. *The Damping Coefficient, k.* This is the coefficient of mechanical resistance in dynes per cm/sec which acts on unit length of the string tending to annul the motion. When the string is a light coated quartz fiber, the air damping is usually so great in comparison with electromagnetic damping that no very great error will result from treating the problem as if the total damping followed the same law as the air damping. This was assumed for simplicity in Part 1, but the theory there developed cannot be applied without restriction to the motion of a string which is a good conductor if the resistance of the external circuit is small. See Section VII.

As mentioned in this Section, §2, k is not really a constant, though in a restricted sense it may be so regarded. It may be determined from the ratios of the successive amplitudes of a train of damped oscillations, the equation to determine k for the oscillatory condition being, $k=4m\lambda'/\tau_k$, for the development of which see Appendix B. If the string cannot be made to oscillate, determine τ_0 from a critically damped deflection, when k is given by $k=4m\pi/\tau_0$. It will be observed that m enters both equations. The real significance of k is in the damping $k/2m$ and experiment has shown this to be constant. If we introduce in either of these equations the true value of m we get a value of k corresponding to a state of motion such that not enough air is dragged with the string to affect the value of m sensibly. On account of the peculiar compensatory relation between k and m, we may use the true values in computation and predict the behavior of the galvanometer correctly at any tension because the changes are always of such a nature as to preserve the constancy of the ratio. The dimensions of k are $[MT^{-1}]$. The damping is of dimensions $[T^{-1}]$, that is, the damping is a time. It is the reciprocal of the time in which oscillations die away to $1/e$ of their initial value, or in which a critically damped deflection dies away to $2/e$ of its initial value.

SECTION 4. EFFECT OF PERFORATION OF POLEPIECES ON THE SENSITIVITY OF THE STRING GALVANOMETER

1. *Theoretical Considerations.* It has been a generally accepted view that weakening the field at the middle region must result in a significant diminution of the sensitivity of the galvanometer. This view seems as a result of our common notions to meet with instinctive approval. The

writer never questioned it until in looking over some of the work in Part 1 several months after its publication, attention was arrested by Eq. (23), p. 156. If L' is the steady current deflection produced by the current, I, in a string immersed in a perfectly uniform field, then if everything remains the same except that the string is clamped fast at the points $5l/12$ and $7l/12$, this equation states that the new deflection due to the current I, is $L_2 = L'/36$. The deduction of Eq. (23) in Part 1 leaves something to be desired in the way of logical rigor, but it can easily be improved. From Eq. (6) we readily obtain $L' = BIl\tau_0^2/32m$. The only factor in this expression which will be altered by change in the length of the string is τ_0. The period of a vibrating stretched string is $\tau_0 = 2l\sqrt{m/T}$, from which it is evident that τ_0 varies directly as l. If the length be shortened to one sixth, the natural period becomes $\tau_0/6$ and the square of the natural period becomes $\tau_0^2/36$, whence it follows directly that $L_2 = L'/36$. Now if the field in the middle sixth be conceived to vanish entirely, the whole string being now free to move, it will be acted on by electromagnetic forces at all points except those lying between $5l/12$ and $7l/12$. The tension is uniform throughout and is taken as being so great that the increase of tension with deflection is negligible. The conditions at the points $5l/12$ and $7l/12$ are then entirely unaffected by the absence of electromagnetic forces over the middle sixth and in consequence the region from zero to $5l/12$ and that from $7l/12$ to l will be deflected to exactly the same places that they would be if the field were uniform throughout. The middle sixth then contributes only $1/36$ of the entire deflection of the middle point and if the field vanished altogether in the middle sixth the deflection of the middle point would still be $35/36L'$, or $.972L'$. In other words the steady current deflection will have been reduced only 2.8 per cent by the vanishing of the middle sixth of the field. Now in an actual galvanometer the field strength in the region of the perforation is by no means zero, but may be as great as half the maximum.

If this deduction rests on a secure physical foundation it is evident that the height of the pole tips may be reduced much below the dimensions of Type B without material effect on the sensitivity. If, for example, the ratio of total height to diameter of perforation be made 3:1, the sensitivity will be reduced about 11 per cent, assuming zero field strength over the middle third. This points to the possibility of a material reduction in the size and weight of the galvanometer without much sacrifice of sensitivity.

2. *Consideration of Experimental Data.* As soon as this implication of theory was realized, all available experimental data which might serve as a check on it were carefully studied. The effective field of the Columbia University copy of the Leyden Model was carefully determined some ten years ago by the heavy wire method described in Section 2, §1. For comparison there was also available a series of determinations of the maximum field strength of this instrument measured with a ballistic galvanometer and exploring coil. The accompanying table gives a series of values by each method.

TABLE 1. *Comparison of maximum and effective field strength. Columbia University copy of Leyden Galvanometer*

Exciting current	Maximum Field, Ballistic Galv.	Effective Field, Heavy Wire
2 Amperes	17,100 gauss	16,800 gauss
3 "	19,700 "	19,400 "
4 "	20,800 "	20,700 "
6 "	22,000 "	21,900 "

Bearing in mind that the field is of considerable strength in the region of the perforation, whose length in this galvanometer is about one tenth of the height of the polepieces and that the possible error of determination of the effective field by the heavy wire method is one per cent while that of the ballistic galvanometer method as used in this work was nearly two per cent, the correspondence between the two sets of observations is such as to suggest that the theoretical conclusion is not far from the truth. One experimental observation seems to be in distinct contradiction. Einthoven (18), pp. 506–07, has calculated the strength of the effective field of his instrument from the difference between the motions of the middle point of a silvered quartz string on open and on closed circuit. He obtained the result that for a maximum field strength of 22,500 gauss, the corresponding effective field strength was 17,600 gauss. In working up the results of this set of observations it was assumed that the shape of the string when deflected by steady current is half of a sine curve.[19] It is also assumed that the difference in rate of decay of the motion of the middle point of the string on open and on closed circuits is proportional to the average velocity of all points of the string, or what amounts to the same thing,

[19] (18), p. 504, Einthoven says, "When the middle of the string moves with a velocity, v_1, one may put for the average velocity of all parts of the string $2/\pi v_1 = .637 v_1$. Now $2/\pi$ is the average value of a sine curve between the limits zero and π, so that it is evident a sine form is assumed.

that the electromagnetic damping at every point is proportional to the velocity as is the air damping. At the time this work was published Einthoven regarded his results in the light of a theory which assumes the motion of the string similar to that of a system with one degree of freedom. This is evident from his use of an ordinary differential equation to define the motion.[20] The assumption of a sine shape for the string follows naturally from this theory. Although the results of this simpler theory often lead to conclusions very little different from those reached by a more general analysis, it is not altogether safe to follow it when the quantities in question are small. Moreover, the equations which apply to the electromagnetic damping were not developed until eight years later.[21] The equations upon which Einthoven's calculation was based[22] were developed in connection with the theory of torsional instruments. These (unless the suspension is bifilar) are systems with one degree of freedom and the electromagnetic damping is strictly proportional to the angular velocity of the system. The theory of these instruments is so well known that it has been quite common to apply to the string galvanometer ideas which have become familiar in connection with torsional instruments. Crehore was similarly misled[23] in the treatment of electromagnetic damping. In the string galvanometer the velocity varies from point to point along the string, being greatest in the middle, while *the current generated by its motion is the same at every point of the string*. The electromagnetic damping is therefore identically the same at every point of the string. When the electromagnetic damping is appreciable the motion of even the fundamental mode becomes complicated, requiring for its expression a correspondingly less simple mathematical function (21, pp. 785–86). The electromagnetic damping of the partial of order s is $1/s$ of the magnitude of the electromagnetic damping of the corresponding amplitude of the fundamental (see Section 7). It might be possible by numerical substitution in Ornstein's equations to evaluate the true effective field from observations of the motion of the middle of the string, but without doing so it is evident that calculation of the results on the assumptions used by Dr. Einthoven must have led to a value for the effective field less than its actual value so that the result of this experiment cannot be regarded as necessarily in serious conflict with the theory of (1).

[20] (18) p. 494.

[21] (19), see reference to the work of L. S. Ornstein in Section 7.

[22] Taken from Kohlrausch, Lehrbuch der Praktischen Physik, 18, p. 480 and 494.

[23] 20, p. 213. See also Section 7.

3. *New Design, Type C.* Having become convinced of the probable soundness of the conclusions in §1, the writer was encouraged to design a new Einthoven galvanometer of very much smaller size than any hitherto described. By reduction in height of the pole tips it became possible to concentrate the windings without making the resistance of the field coils excessive. In this way the leakage is somewhat diminished and the bulk of it confined to the immediate neighborhood of the instrument, an obvious advantage. An experimental model of this galvanometer has been completed and its performance has been found to be in agreement with theoretical predetermination. Its weight is 5.8 kilos which may be compared with 18.15 kilos for Type B and 100 kilos for Type A. A further note describing this instrument will follow in the immediate future.[24]

SECTION 5. LIMIT OF SENSITIVITY AT WHICH THE DIFFERENTIAL EQUATIONS OF MOTION CEASE TO APPLY

It was pointed out in Part 1, p. 150, that the equations there developed will not apply after the tension becomes non-uniform. It is of interest to know at what order of sensitivity this limitation begins. To this an entirely unequivocal answer cannot be given without full development of a theory applying to the string when the tension is not uniform. For the present we may gain some practical insight into the matter by subjecting available experimental data to numerical computation. In the first place it is evident that if the string be slackened so far that it is not stretched beyond its "natural" length, the tension will be due to gravity only and at the upper end will be equal to mgl dynes. If the origin of Y be taken at the upper end of the (vertical) string, the tension at any point, P, whose ordinate is y, is given by $T = mg(l - |y|)$.[25] However much tension is put on the string, this non-uniform tension due to gravity is always present so that uniformity can be approximated only by making the impressed tension so large in comparison with the gravity effect that the latter becomes negligible in comparison. The numerical data which we are about to consider have been obtained from a galvanometer of Type B with a string 6.7 cm long. The linear density, m, for this string has been determined to be 2.42×10^{-7} gram/cm. (Appendix D.) In a particular experiment (Appendix C), the value of τ_0 was found to be 9×10^{-4} second. From

[24] This instrument was described and shown at the 137th meeting of the American Physical Society at Montreal, February 26, 1926.

[25] See E. J. Routh, Advanced Rigid Dynamics, 5th Ed., p. 379.

Part 1, Eq. (5), we have $T = 4l^2m/\tau_0^2$. From this and the numerical values given, we readily calculate $T = 53.7$ dynes as the tension at which this determination of τ_0 was made. This tension was sufficient to make the motion distinctly periodic, several highly damped oscillations taking place. The value of mgl, the limiting tension, we also readily find to be 1.59×10^{-3} dynes, from which we see that the tension in the periodic condition was some 34,000 times as great as the tension at the upper end when the string is fully slackened. It may be mentioned that the tension 53.7 dynes is about one tenth what was found to be a destructive tension for this string. This string was originally 7 cm long. In an attempt to make its motion as highly periodic as possible a tension of some 500 dynes was put on it, but before all the desired records were obtained it became nonconducting. It was resoldered and its length reduced 3 mm in so doing. It may be noted that 500 dynes would be a perfectly safe tension for this string so far as the tensile strength of the quartz is concerned, but the limit is determined by the tenacity with which the gold coating adheres to the quartz.

At critical damping the undamped period of this string was found to be 2.4×10^{-3} second (Appendix C). A calculation similar to the preceding gives for the tension when just aperiodic, 7.54 dynes. This is 4,700 times as great as mgl. The latter may surely be assumed negligible in comparison. Presumably a variation of one per cent in the tension would not vitiate the equations and we may inquire at what sensitivity this string would be under a tension equal to $100\ mgl$. We have (Part 1, p. 137) $\tau_0 = 2l\sqrt{m/T}$. Put in this equation $T = 100mgl$, using the values of m and l given above and taking g, the acceleration of gravity, as 980. We get for τ_0 the value 1.654×10^{-3} second. Now it is evident that if we alter the tension on a string and also alter the current so that the field remaining constant, the deflection is kept constant, then the product of the current by the square of the natural period will remain constant (Appendix C). If we know this product for a given field and string at any tension, we may infer either term at any other tension provided we know the other at the new tension. In the experiment for which the value of the tension was calculated to be 53.7 dynes, $\tau_0^2 I = 8.6 \times 10^{-9}$. If we square the value of τ_0 obtained above for $T = 100\ mgl$, and divide the number 8.6×10^{-9} by the result, we shall obtain the value in cgs units, of the current which will deflect the string under tension $100\ mgl$ the same amount that the current I deflected it when under tension 53.7 dynes. The square of 1.654×10^{-3} is 2.736×10^{-4} and $(8.6 \times 10^{-9})/(2.736 \times 10^{-4}) = 3.14 \times 10^{-5}$ cgs units of current, or 1.04×10^{-4} ampere/cm. On the assumption that one per

cent variation in the tension is negligible, the equations still hold at this tension and the string is not yet like a vertical chain. Now it is of considerable interest to note that at just about this sensitivity a critical change in the behavior of the galvanometer is observed. At tensions slightly greater than 100 *mgl* the range of the linear deflection law is found to be smaller than it is at still greater tensions. This is no doubt due to the fact that the tension has now become so small that the increase with deflection is no longer negligibly small in comparison. A slight movement of the tension screw now affects the sensitivity markedly. As the sensitivity approaches that for which we have calculated the tension as of the order of 100 *mgl*, there is usually a slight shift of zero and focal plane, the sensitivity changes less rapidly for a given movement of the tension screw and the deflections again become proportional to the strength of current. The most reasonable explanation of these observations seems to be that the tension is now of the same order of magnitude as the internal stresses in the quartz core (metallic wires show the same phenomena, probably for the same reason), which is no longer constrained to remain in a straight line and assumes slight curvatures according to the magnitude and distribution of the stresses. Aside from stresses in the string itself, there may be introduced lateral and torsional stresses in the soldering of the ends of the string to the metal tips. This has been noticed by Einthoven (7). In making the strings one often discards quartz fibers which are distinctly curly and presumably even the best of those retained have something of this tendency. A string slackened until these stresses have been relieved is still under approximately uniform tension in something like the manner of a spiral spring of very coarse pitch. The change in length due to deflection may now occur by slight straightening of the stress curls without materially affecting the general uniformity of tension, hence the restoration of the linear law of deflection.

Since the equations show that the sensitivity varies inversely as the tension, we may infer that at a tension of 1000 *mgl* this string would have a sensitivity of about 10^{-7} ampere/cm. This may be of some interest as being about the sensitivity which is standard for the taking of electrocardiograms. With the particular galvanometer and string to which these figures refer, a sensitivity of 5×10^{-10} ampere/cm was easily reached. A tension of $10mgl$, which would mean non-uniformity of tension to the extent of ten per cent, would correspond, if the usual equations still applied, to a sensitivity of 10^{-9} ampere/cm. From this it seems sufficiently evident that at the actual experimental limit of sensitivity the tension is distinctly non-uniform and that the behavior

of the galvanometer cannot be calculated with any certainty in the neighborhood of this limit by use of the theory for uniform tension.

In concluding this Section it may be mentioned that Wertheim-Salomonson has published a paper (21) in which he finds a mathematical expression for the sensitivity of the string galvanometer when the tension is relaxed as completely as possible. He treats the string in this condition by means of equations given by Lord Rayleigh for an elastic bar clamped at one end. It is open to question, however, whether it is admissable to regard the string in this way since it is fastened at the bottom and one cannot neglect rigidity at the bottom if the string is treated as a bar. He further assumes that since the sensitivity under uniform tension is proportional to the square of the natural period it is so also when it behaves as an elastic bar. This would seem to require proof. It is to be remarked, however, that the numerical results to which this theory leads agree fairly well in order of magnitude with the maximum sensitivities actually obtained with the best instruments hitherto constructed.

It seems to the writer that it would be, perhaps, more nearly in correspondence with actual conditions to treat the relaxed string by the equations for a rope or chain hanging under gravity.

SECTION 6. THE PERFORMANCE OF THE STRING GALVANOMETER

Much regarding performance may be inferred from the theory, but a prospective user of the Einthoven galvanometer will often wish specific information regarding results which have actually been obtained with available types of the instrument. In the papers cited Einthoven has given such data for the Leyden Model, and Type A described in this paper corresponds very closely in its properties and performance with his original instrument. The properties of any particular instrument will vary with the constants of the string which happens to be in use so that the values given should be regarded as representative only.

The length of the string in Type A is usually 13.5 cm and the resistance of these strings may be less than 1000 ohms and as great as 30,000 ohms. Usual resistances of strings for physiological and clinical use range from 3000 to 10,000 ohms. In the Columbia galvanometer a string 13.5 cm long and .0018 mm in diameter was found to be critically damped on short circuit when the current sensitivity was 2×10^{-7} ampere/cm with a magnification of 920 diameters. The resistance of this string was 8000 ohms. The value of τ_0 for this string at critical damping was .004 second. Substituting in Eq. (11a), Part I,

τ_0 for t, we readily find that a steady current deflection will be nearly 99 per cent complete in a time equal to the natural period if the damping is just critical. If the instrument is to be used for measurement of periodic currents the natural period and steady current sensitivity in connection with the curve on p. 143, Part 1, will give a good index of the performance which may be expected. It is to be remembered in this connection that the response to a complex periodic current may differ slightly from what might be inferred from this curve since the curve represents the response of the fundamental mode only and the partial modes are somewhat underdamped. For critical work it would probably be well to prepare an experimental calibration curve by test with known alternating current. For most work it is best to use the instrument with the fundamental mode just critically damped. Strings whose mass differs from the one above referred to will give somewhat different results. For most work it is well to have them of very small diameter. The above data are for field strength of 20,000 gauss. With strings 13.5 cm long the period cannot be maintained at .001 second without danger of injury unless the diameter is very small. On the other hand it is advantageous to have the properties of the string such that critical damping will occur at about the tension which gives suitable sensitivity for the work in hand and this may sometimes require a heavier string. At a sensitivity of 10^{-8} ampere/cm the motion will be overdamped for almost any light quartz string in any good string galvanometer. As sensitivity is still further increased by slackening the string, the overdamping increases and the motion ultimately becomes very slow at the maximum sensitivity so that ten or fifteen seconds may be required to attain 99 per cent of the steady current deflection with the string as slack as possible. Theoretically the instrument could be much improved by having the string in an evacuated space at these high sensitivities. The experimental difficulties are considerable and I am not aware that anyone except Dr. Einthoven has had any extensive experience in the use of the galvanometer under this condition. As his work is not yet published I do not feel at liberty to discuss it. It may not be improper, however, to mention the fact that difficulties have been encountered in attempting to carry out this apparently simple procedure.

With the Leyden Model Einthoven obtained sensitivities of the order of 5×10^{-11} ampere/cm. At this sensitivity the stability of zero is remarkable. When using the galvanometer at such high sensitivities it is advisable to protect all parts of the circuit by electrostatic screening and it is often advisable to earth one end of the string. Mr. Louis G.

Pooler, of the Department of Physics, Columbia University, having occasion to work with a Type B galvanometer under circumstances which made earthing undesirable, found that he could prevent the building up of any considerable charge on the string by putting into the lower string cap a small tube containing the active deposit left by radium emanation. These tubes are easily obtained from any clinic where radium emanation is in use. Earthing is usually preferable, however. In case a string becomes charged it usually is attracted to and adheres to one of the microscope objectives unless it is under great tension. The procedure when this occurs is to increase the tension, then cautiously back away the lenses, watching the middle of the string through an opening usually provided for the purpose. Sometimes it is necessary to remove the string and replace it. This may injure the coating, but usually it does not. It is better to arrange the circuits so as to avoid these accidents. They are more likely to occur in winter when buildings are heated and dry. A metallic matting which may be earthed and on which the operator stands is often helpful. If a spark passes from the string to the microscope mounting the coating is usually ruined. Wiping the lenses is seldom necessary. If it has to be done the galvanometer should not be used with slack string for several hours. It might be supposed that deposition of a film of moisture on the lens by breathing on it would discharge it, but the writer has seen disturbance from this cause persist for several hours. The effect may be related to the phenomenon of residual charge well known in condensers. It must be remembered that the force required to deflect a slackened quartz fiber is extremely small. When greatly slackened the string is easily disturbed by air currents and before attempting measurements with it in this condition it is well to test the means provided for protection and supplement them if necessary.

Type B was not originally intended to be used with slack string; but no particular difficulty was experienced in reaching with one of these instruments a sensitivity in the neighborhood of 10^{-9} ampere/cm, the magnification being 1000 diameters in this experiment.

At critical damping its period will be about half that of Type A with a similar string, but at equal sensitivities the periods are of the same general order of magnitude. On account of the shorter string it can be operated at $\tau_0 = .001$ second with the average commercial string. With a magnification of 1000 diameters Type B instruments have a linear deflecton law for about 8 cm either side of zero. One such instrument in the writer's laboratory gives proportional deflections for 10 cm either side of zero when the string is slack. The large Edel-

mann instruments with strings supplied by the maker usually give proportionality for three cm either side of zero at 1000 diameters magnification. With a very good quartz fiber the writer has secured proportionality with this instrument for 4 cm, the sensitivity being 10^{-7} ampere/cm. Most commercial instruments have been handi-

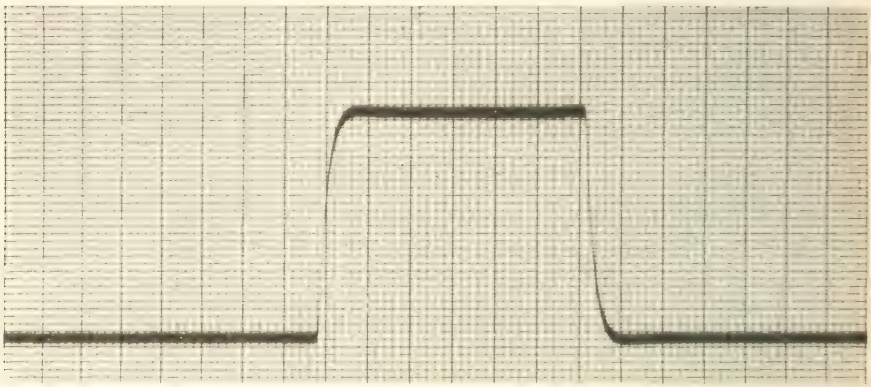

FIG. 7. a. *Damping barely less than critical. 6.3×10^{-7} ampere. The time co-ordinates are .001 second for one small division. $\tau_0=.0035$ approximately.*

capped by a very limited proportionality range. When the polepieces are not carefully made and the field is not uniform the deflections may become greater with increasing equal increments of current on one side of zero and rapidly less on the other side. This the writer has seen in an instrument whose polefaces were measurably inclined laterally. The

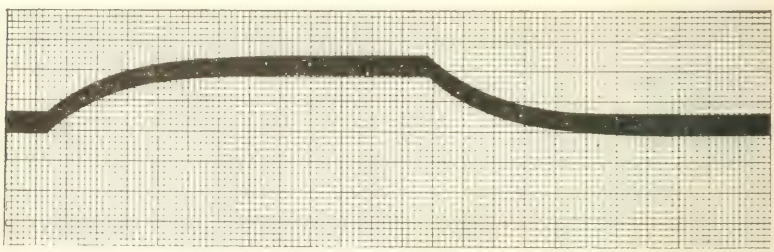

FIG. 7. b. *Damping very much greater than critical. 10^{-9} ampere per cm. Time co-ordinates .04 second for one small division. The magnification is 920 diameters in both. They were not made with the same string.*

weaker the field and the lower the magnification the smaller the linear range will be. For a given volt sensitivity it will be less the higher the resistance of the string, unless it be used quite slack, and less for a very heavy string than for a light one. Below are reproduced some photographic records which with their legends will serve to give further

information as to the average performance of these instruments. It will be realized that they are at their best in making measurements at moderate frequencies where considerable sensitivity is required. The writer constructed an instrument with string one cm long with which a natural period of .0001 second was easily attained, the sensitivity at that period being 10^{-4} ampere/cm, but air damping was not nearly sufficient to suppress the oscillations and the original plan to work with

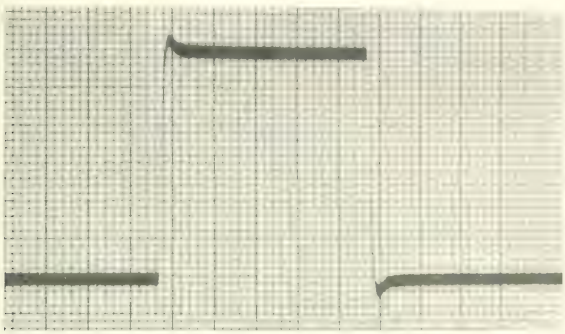

FIG. 7-c. *Showing the speed of deflection increased with moderate under-damping. Time, .001 second per small division.*

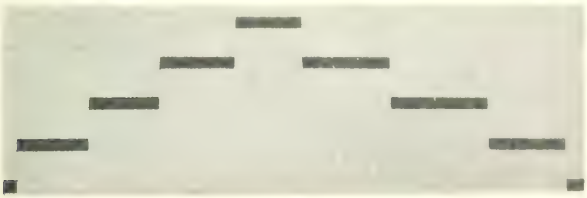

FIG. 7-d. *Damping critical. Shows proportionality of deflection to equal increments of current and constancy of zero. Time, .04 second for one small division. The sensitivity is approximately 10^{-7} ampere/cm.*

low resistance strings so as to secure high electromagnetic damping was abandoned for reasons which will be discussed in the next section. By the use of liquid damping this instrument might be made very sensitive but on account of the addition of mass through dragging of the liquid, the sensitivity would be considerably reduced. There are several liquids of suitable viscosity whose refractive indices are such as would make possible the use of immersion lenses with corresponding increase of aperture. Thus by use of strings of small diameter and working at high magnification the loss of sensitivity might be partially overcome

W. F. Einthoven has described (14) a string galvanometer using strings a fraction of a millimeter long. The air damping is reduced to a minimum by putting the string in vacuum and the instrument is used tuned to resonance with the frequency of the current. It was used as a receptor of radio telegraphic signals and at the rapid rate of oscillation its movements would not be resolved on a rather slowly moving photographic paper so that the record appeared to consist of "disappearances" of the string, giving the effects of dots and dashes. It has been possible to attain natural frequencies as high as 300,000 cycles per second with this instrument.

SECTION 7. THE EFFECT OF THE CIRCUIT

In Part 1 the equations of motion were developed on the tacit assumption that the effect of the external circuit is negligible. Though this is never entirely true, it is so nearly true in the circumstances in which the galvanometer is usually employed that it was thought advisable to develop the theory in the form which seemed best adapted to direct attention to the more important features without undue complication. In the usual applications the resistance of the string is large and the external resistance is often large also. Under these circumstances the damping will be almost entirely due to the air. The inductance of the circuit is always small in the physiological applications and with a circuit of high resistance inductance would have to be large if its effect on the time of deflection is to become comparable with the mechanical lag of the string. If the string galvanometer comes in time to be applied to the measurements of physics and engineering, circumstances will likely arise in which the effect of the circuit can no longer be neglected. Since the war some of the sound ranging galvanometers mentioned in Section 3, §4, have been applied to some extent to current measuring problems. Trowbridge (22) has suggested that similar instruments may be used conveniently instead of the more usual oscillographs. It would seem fairly obvious to try to extend the range of such an instrument with vacuum tube amplifiers, and this has been done. In these instruments the electromagnetic damping is large compared with the air damping, exactly the reverse of the condition which obtains in the sensitive galvanometers with quartz strings. In the use of such instruments the effect of the external circuit cannot be neglected if the work in hand requires actual current measurement rather than the indication of time when a current begins as in the sound ranging application.

The first investigation of the circuit equations of the string galvanometer was made by Crehore (20). His work led Ornstein (19) to undertake a study of the subject. Ornstein's paper (in English) has apparently attracted little attention probably because it appeared when events at the beginning of the war were occupying attention and perhaps in part because of limited distribution of the Amsterdam Proceedings. This paper, which was unknown to the writer when Part 1 was written, deals with the motion of the string under electromagnetic damping. The treatment is original and rigorous and is undoubtedly the most compact and general exposition of string galvanometer theory available. It is, however, so much condensed that a reader of moderate mathematical ability will find it somewhat difficult to follow. In referring to Crehore's deduction of the circuit equation, Ornstein (p. 784) remarks, " . . . . not entirely exact energetic considerations underlie this deduction." This seems to refer to the following statement in Crehore's paper (l.c., p. 213), "Since the back electromotive force is proportional to the velocity, we may write, $e' = k_s' \varphi_s$, where k_s' is a constant quantity . . . . ." As mentioned in Section 4, §2, ideas derived from consideration of the moving coil galvanometer seem to have led many people to look upon the electromagnetic damping of the string galvanometer as simply proportional to the velocity. That this cannot be so was first pointed out to the writer in conversation by Professor H. W. Webb. Ornstein shows (l.c., p. 785) that for the partials the electromagnetic damping will be less the higher their order. This appears from his analysis, but is evident from physical considerations without mathematical development, a fact to which he draws attention. The adjacent segments of a string vibrating in parts move in opposite directions and generate emf's of opposite sign, so that of a train of odd partials *only one segment* contributes to electromagnetic damping. In Part 1, p. 160, reference is made to the motion of a metallic wire under electromagnetic damping. It was observed that when the "fundamental mode"[26] of this wire was more than critically damped, the third partial was still strongly in evidence. In Fig. 8 are represented two records of the motion of this wire. In one it is oscillating on open circuit and the decrement is small. In the second record the "fundamental" is somewhat overdamped. The third "partial" can be distinctly seen and in the original record the fifth can be distinguished also. At the time of writing Part 1 no satisfactory explanation of the

[26] Bearing in mind the results of Ornstein's paper it is perhaps unfortunate to speak of a fundamental mode in the usual sense. Something approximating it exists, however.

persistence of these partials could be advanced, but in the light of Ornstein's results exactly this would be expected.

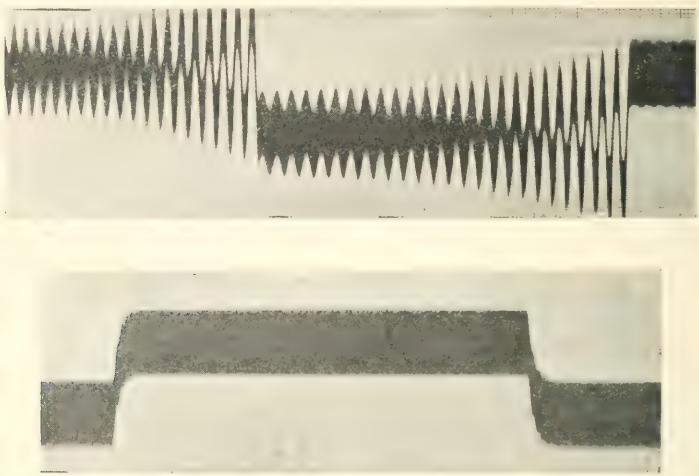

FIG. 8. *Motion of Duralumin wire.* a. *With air damping only.* b. *Heavily shunted. Time co-ordinates .001 second for one small division. Note effect of partials on the wave crests in* a. *Also the appearance of the third partial on the curves of growth and decay of the overdamped fundamental in* b.

Since with predominant electromagnetic damping the whole force available for damping the total motion is proportional to $\sum_{s=1}^{\infty} 1/s$ of all the emf's generated, it is obvious that the remarks as to resonance of the partials, Part 1, p. 163, will not apply in these circumstances. It will also be evident why the cruder galvanometers using heavy wires as strings cannot be made equivalent to galvanometers using light coated quartz fibers by the simple expedient of applying amplifiers. If, however, the string is damped with liquid and the external resistance so adjusted that the electromagnetic damping is small in comparison with the fluid damping, the heavy wire instrument may be made more serviceable for measurements of precision. If used with magnetic damping only, care must be exercised in interpretation of the curves.

It may be here remarked that the bifilar oscillograph, though usually treated theoretically as an instrument with one degree of freedom, in reality resembles very much a string galvanometer of low resistance with some added complication due to the coupling of the strings at the middle and loading them with the mirror. These instruments are usually used with fluid damping and at a natural period much shorter than that of the fundamental of the periodic current to be measured. It seems possible that in some applications the motion of the instrument may

be measurably affected by the occurrence in the impressed current of a component in agreement with one of the partial modes of the oscillograph system.

SECTION 8. ACCESSORIES TO THE STRING GALVANOMETER

1. *Illuminating Systems.* While the string galvanometer may be used subjectively and the motion of the shadow observed with an ocular provided with a scale, its peculiar field of usefulness like that of the oscillographs is in the investigation of phenomena whose variations are too rapid to follow with the eye. For photographic registration or for the use of a rotating mirror or similar device the instrument must be used as a projection microscope and some means must be provided for illumination. For many purposes a wire filament gas filled incandescent lamp is satisfactory. A simple condenser is necessary and it is advantageous to use a pair of lenses in order to diminish the spherical aberration. Small motor headlight lamps may be used, but they usually have a double filament. A single coil is preferable. It should be centered and then put out of focus until the field is uniformly illuminated with overlapping circles of diffusion. Definition of the string shadow will be poor unless the source of light is centered. Such a lamp may be used at normal voltage for recording the electrocardiogram, the magnification being about 500 diameters and the speed of photographic registration 25 mm/sec. By employing temporary overvoltage higher magnifications or higher speeds may be used. For very short exposures it is possible to secure quite satisfactory records on bromide paper at three meters a second. These lamps are inexpensive and have the advantages of requiring no attention after initial adjustment and consuming little current.

For still higher speeds up to ten meters a second the carbon arc is available. A smoothly operating automatic projection lamp using cored hard carbons is most convenient. If a really good automatic lamp is not available a hand feed lamp is preferable to a poor automatic. It may be operated on as small a current as 5 amperes, but the writer prefers to use larger carbons and more current, ten to fifteen amperes. The larger crater is steadier and there is less likelihood of its wandering enough to spoil a record. The best system of accessory lenses for use with an arc lamp is that described by Einthoven (*Z.*, p. 510). The condenser is a spectacle lens 3.5 cm in diameter and there is a second lens and two iris diaphragms. The entire arrangement is inexpensive and can easily be improvised in any laboratory. Einthoven arranges to illuminate the string with diffusion circles and so avoids sharp light

and dark bands across the records due to slight changes in the local temperature of the crater. This is desirable since, as will be explained presently, similar shadows are used to make the time record. A very great advantage of his system of illumination is that one of the iris diaphragms may be used to regulate the intensity of illumination, the other controlling the size of the illuminated field. These small lenses entirely fill the aperture of the microscopes. When Nernst lamps were available they were used extensively with string galvanometers. Tungsten arc lamps are in use to some extent. Aside from the initial expense and their rather short life they should be satisfactory.

2. *Photographic Registration.* For registration at slow speeds as in clinical electrocardiography, a variety of recording instruments have been developed by the makers of apparatus. Most of these have a magazine of film or photographic paper so that long continuous records may be made or they may be cut off at any desired point. The exposed paper usually accumulates in a box which may be removed and taken to a dark room for development. Others, as that devised by Mr. Lucien Bull, of the Institut Marey (made by Boulitte) carries the film or paper on a revolving drum similar to those used with oscillographs. The drum type is best for short records at high speed and for records of greater length at high speed one may use a drum which travels along a spiral. An excellent instrument of this type has been described by Eckhardt (23). The writer has used for many years a recorder described by Einthoven (24), adapted to all speeds from a millimeter a second to a meter a second. Apart from the fact that it is bulky this is a perfectly satisfactory instrument. It uses photographic plates. A variety of plate recorders have been devised in which the plate falls, uniformity of motion being attained in some by the principle of the Atwood machine and in most by damping. The damping is often obtained by using a piston displacing oil or air through a precision cock. Edelmann built a falling plate recorder in which the damping was obtained by rotation of a copper disk between electromagnets. An excellent discussion of the whole subject of photographic registration will be found in the article so entitled by the late Professor S. Garten in Tigerstedt's Handbuch der Physiologischen Methodik, (25). At the end of this article is a bibliography of 111 articles which probably includes everything of consequence in this field up to 1910.

Of the various available photographic materials paper is by far the cheapest and bromide paper can now be had which gives excellent results at magnifications of 500 diameters and speeds of two meters a second with a wire filament lamp. Slower papers are also available.

Reproduction of records is less convenient than with film or plates and neither film or paper is as convenient as a glass plate when precise measurements are to be made under a microscope. Plates can be had in every variety of speed and grain. They can be used in the falling plate type of recorder up to speeds of two meters a second and probably faster. Fast records of condiderable length require paper or film. Film may be had in long rolls either of the usual high speed emulsion, or with a slower emulsion such as is used for motion picture positives and called "positive film." This is excellent for sharp contrasts at slower speeds. Fast film will give good records at five meters second with a magnification of 1000 diameters using a carbon arc lamp and legible records at ten meters a second.

Most inexperienced persons use too much light and too sensitive emulsions for photographic registration. The records should have a maximum of contrast and this is best secured by using the slowest and finest grained emulsion and the smallest amount of light which will give results. The best optical definition is secured by using a source of high intrinsic brilliancy such as a carbon arc and cutting down the aperture with a diaphragm as in the illumination system of Einthoven. Since even the best objectives are imperfect in color correction it is well to ascertain by trial where to focus the subjective image sharply in order to get the best photographic definition. For a discussion of this matter and suggestions as to alignment of the optical systems reference may be made to a paper of Einthoven (26) on simultaneous records with several galvanometers.

During the war a detachment of engineer soldiers under the writer's direction constructed a number of photographic recorders of the magazine type with a device for automatic development and fixation. The records were developed and fixed in ten seconds and the device functioned with great reliability. Trowbridge has recently described a similar device for use at greater speeds (22). Military experience proved that photographic registration with suitable equipment is not only not difficult, but may offer the simplest means all things considered of solving a problem of graphic recording.

3. *Recording of Time Co-ordinates.* In the usual commercial application of the oscillograph it is customary to infer the time relations of the record from the average R.P.M. of the drum. If measurements of high precision are to be made this is unsatisfactory, not alone because the rate may change, but because the photographic material in the process of development, washing and drying swells and shrinks. The most

recent information on the shrinkage of film is to be found in a paper in this Journal (27) by Curtis. In order to control with certainty the actual velocity of the record and at the same time to provide a reference system small enough to make the shrinkage negligible, Garten (28) devised the plan of photographing simultaneously with the record a system of rectangular coordinates. The space coordinates are generated by a series of lines ruled on the back of the cylindrical lens of the recorder. The time coordinates are shadows cast by the spokes of a wheel which rotates in such a position as to cut off the light at regular intervals. By making every fifth spoke thicker each fifth line is heavier. For controlling the speed of the wheel at a great variety of speeds Einthoven has described a very convenient device (29). For speeds not greater than one or two hundred mm/sec, the phonic wheel of LaCour (30) and Lord Rayleigh (31) serves very well. It was first applied for this purpose by Bull. The writer has used since 1912 a regulator described by Giebe (32) which was constructed at the Reichsanstalt, after a suggestion of Helmholtz, for controlling the speed of a rotary commutator used in measurement of capacity by the Maxwell bridge method. With two interchangeable regulator spindles this provides a large range of time intervals up to 1000 shadows a second. A modified form gave satisfactory results at 40 R.P.S. which with a 50-spoke wheel gives 2000 shadows a second. The co-ordinate network on the records published in this paper was recorded with this apparatus.

For the measurement of short time intervals by photographic methods with very high precision the tuning fork itself may be made to interrupt the beam of light in the manner described by Curtis and Duncan (35). For data on the accuracy of electrically driven tuning forks used in such measurements, see a paper by Dadourian (36).

APPENDIX A

ERRATA IN PART 1

At the beginning of this paper is a note calling attention to a defect in the statement of Eqs. (10) and (16) of Part 1, which was kindly brought to my notice by Professor H. W. March. In Eq. (10) the sign of summation should not have been used since its customary meaning would imply that all of the infinite number of solutions exist simultaneously, whereas they actually exist one at a time. The complementary function corresponding to any critically damped partial of order $s=lk/2\pi am$ is given by Eq. (10) with the sign of summation omitted. As a is altered by change of tension, $lk/2\pi am$ becomes equal

to each of the odd integers, s, successively. A similar remark applies to the second sign of summation in Eq. (11).

In Eq. (16) the limits of the sum should be from $s=1<lk/2\pi am$ to $s=(2n+1)<lk/2\pi am$. This equation will then apply to all modes of the string's motion for which s is less than the number corresponding to the order of the mode which is critically damped. For all orders of s above the one which is critically damped the motion is damped by Eq. (8). These corrections do not affect in any way the results of Part 1 since the interpretation put on these equations was that here stated as will be evident from remarks on page 159.

The following additional errata have been noted:

Page 144, fourth line above Eq. (14) read, *sth.* partial *is* critically damped. . . . .

Page 159, 4th line below Eq. (35) the statement that the fundamental decays at the same rate as the oscillations is not rigorously correct. The law of decay of the fundamental is given by Eq. (15a), p. 147.

Page 170, line 28, Eq. (III) should read, $u=e^{\beta t}\ (A+Bt)$.

Page 173, the last equation in Appendix h should read,

$$u=U\left\{\epsilon^{\beta_1 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right)-\epsilon^{\beta_2 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right)\right\}$$

Page 174, the date of Ref. (4) should be 1910.

Appendix B

Damped and Undamped Periods, Decrement and Damping Coefficient, k

1. *Period and Decrement.* When the relation between mass, damping and tension is such that the motion is oscillatory as defined by the complementary function of Eq. (8), Part 1, the damped period of the fundamental mode is easily determined. The string is deflected by steady current and if the effect of air damping alone is to be measured, a photographic record is made of the return to zero when the circuit is opened. At the same time there is registered a record of the time which may conveniently be in thousandths of a second. The period of one or several oscillations is then compared with the time scale under a micrometric microscope. From the damped period so determined we require the period the string would have if the damping were removed. The necessary formulas are readily developed from the results of Part 1. Although they are not new, the development, which is simple, will

serve to make clear their meaning. This seems desirable as confusion sometimes arises from the use of formulas based on the half period and others based on the whole period without explanation.

Let $u_1, u_2, u_3, \ldots$ be the amplitudes of successive whole oscillations of a damped train. If there were no partials the ratios $u_1/u_2, u_2/u_3 \ldots$ would all be equal. Einthoven found discrepancies which Crehore referred to persistence of the third partial during the first oscillation. Using a Type B instrument with a string of half the length of the Leyden Model it is possible to get much larger oscillations and in these circumstances the writer finds no discrepancy greater than the probable errors of measurement. The discrepancy observed by Crehore, whose curves were registered in the Columbia laboratory, was always that the first oscillation was smaller than it should have been had the motion been simply exponential. It is possible that some cause other than the persistence of the third partial contributed to increase the discrepancy in these records. At all events I have been unable to make certain of the effect of the partials in the smaller instrument though theory indicates it should be but little smaller than in the larger one.

If τ_k is the damped period, we have $u_1 e^{-k t/2m} = u_2$, from which it follows that

$$\log u_1/u_2 = k\tau_k/2m \tag{1}$$

The second member of (1) is called the logarithmic decrement and is usually represented by λ.

The analytical expression for the fundamental damped period (Part 1, p. 137), is

$$\tau_k = \frac{2\pi}{\sqrt{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} \tag{2}$$

Introducing this value of τ_k into the right hand member of (1), we have

$$\lambda = \frac{2\pi k}{2m\sqrt{\frac{\pi^2 a^2}{l^2} - \frac{k^2}{4m^2}}} = \frac{2\pi}{\sqrt{\frac{4\pi^2 a^2 m^2}{l^2 k^2} - 1}}$$

from which

$$\lambda^2/4\pi^2 = \frac{1}{\frac{4\pi^2 a^2 m^2}{l^2 k^2} - 1}$$

and from this by simple algebraical transformations we readily obtain

$$1+\frac{\lambda^2}{4\pi^2}=\frac{1}{1-\dfrac{l^2k^2}{4\pi^2a^2m^2}} \tag{3}$$

Multiply both terms of the right hand member of (2) by l/π, then

$$\tau_k=\frac{2l}{\sqrt{a^2-\dfrac{l^2k^2}{4\pi^2m^2}}}$$

which is identical with

$$\tau_k=2l/a\sqrt{\frac{1}{1-\dfrac{l^2k^2}{4\pi^2a^2m^2}}} \tag{4}$$

But $2l/a=\tau_0$ (Part 1, p. 137), whence from (3) and (4) we have,

$$\tau_k=\tau_0\sqrt{1+\lambda^2/4\pi^2}$$

For purposes of numercial computation the radical in (5) may be expanded by the binomial theorem, all terms after the second being negligibly small, and we have,

$$\tau_k=\tau_0(1+\lambda^2/8\pi^2+\cdots) \tag{6}$$

The damping of a string galvanometer is always large so that under the maximum tension which will not injure the string there will be only a small number of oscillations before the amplitude falls off to a magnitude comparable with the unavoidable error of measurement. Instead of taking the ratios of the amplitudes of whole oscillations, we may determine the decrement from the ratio of any maximum to the absolute value of the next following minimum. The time of a half period is $\tau_k/2$. Writing for the logarithmic decrement of a half period λ', we have $\lambda'=k\tau_k/4m$, or $\lambda'=\lambda/2$, so that (5) becomes $\tau_k=\tau_0\sqrt{1+\lambda'^2/\pi^2}$ and expanding the radical,

$$\tau_k=\tau_0(1+\lambda'^2/2\pi^2+\cdots) \tag{7}$$

2. *To Determine the Value of the Damping Coefficient, k.*

1. When the motion of the string is periodic.

$$\lambda=k\tau_k/2m, \text{ whence } k=2m\lambda/\tau_k \tag{8}$$

If the decrement is determined for a half period,

$$k=4m\lambda'/\tau_k \tag{9}$$

2. When the motion is aperiodic. We consider the fundamental mode only. The criterion of exact aperiodicity is $\pi a_1'/l = k_1'/2m_1$, whence $k = 2m\pi a_1'/l$. But (Part 1, p. 137), $2l_1'/a = \tau_0$, so that $2a_1'/l = 4/\tau_0$, which gives,

$$k = 4m\pi/\tau_0 \tag{10}$$

APPENDIX C

CONSTANCY OF DAMPING AT HIGHER TENSIONS

Einthoven's experiment discussed in Section 3, §2, has shown that the damping, $k_1'/2m_1$, is constant for a tenfold variation of tension when the absolute value of the tension is small. The following experiments indicate constancy of this ratio from a tension corresponding to critical damping to one approaching the maximum safe limit. The galvanometer used was Type B; length of string 6.7 cm, resistance 18,000 ohms. During the experiments the exciting field current was kept constant. The string was deflected by known steady direct current and the circuit then opened. The curve of return to zero was recorded on plates at one meter/second, with a time scale in thousandths of a second. At the greater tension there were several oscillations. The value of τ_k was determined by direct comparison with the registered time scale under a microscope provided with cross-hairs and a vernier stage. The amplitudes of successive maxima and minima of the oscillations were similarly measured. The ratio for the half period was found to be in one experiment 3.87. Log 3.87 $= \lambda' = 1.35$. $\lambda'^2 = 1.82$, $2\pi^2 = 19.7$, whence $1 + \lambda'^2/2\pi^2 = 1.0925$. The measured value of τ_k was .001 second. By Eq. (7), Appendix B, $\tau_0 = \tau_k \, 1.0925$. This will be the undamped natural period at maximum tension and to distinguish it from the undamped natural period at critical damping we shall add the subscript 1, so that $\tau_{01} = 9 \times 10^{-4}$ second. From Appendix B, (9) and (10) we have $k = 4m\lambda'/\tau_k$ for the periodic condition, and $k = 4m\pi/\tau_{02}$ for critical damping. Between these two equations k and m have presumably varied. We shall assume that over the range of tensions covered by the experiments the ratios k_1'/m_1, k_2'/m_2, are constant and see to what conclusion this assumption leads us. From (9) and (10) we then obtain $\tau_{02} = \pi\tau_{01}/\lambda'$, where τ_{02} is undamped period at critical damping and τ_{01} is damped period in the oscillatory state. Substituting the numerical values, we have $\tau_{02} = 3.14 \times 10^{-3}/1.35$ or, $\tau_{02} = 2.325 \times 10^{-3}$.

From Eq. (6), Part 1, we readily infer that with the same string in the same field, if the tension is varied and the deflection kept constant by simultaneous variation of the current through the string, the

product $\tau_0^2 I$ will be constant, τ_0 here referring to the prevailing tension, i.e., being regarded as a variable. We know the value of this product in the experiment under consideration for the periodic condition. It is $\tau_{01}^2 I_1 = 8.62 \times 10^{-13}$. If we divide this number by the square of the value of τ_{02} which we have calculated for the aperiodic condition, and if our assumption as to k and m is correct we shall obtain the value of the current which will produce the constant steady deflection U at the tension for which the string will be critically damped. We find $\tau_{02} = 5.41 \times 10^{-6}$ and $(8.62 \times 10^{-13})/(5.41 \times 10^{-6}) = 1.59 \times 10^{-7}$ cgs units of current or 1.59×10^{-8} ampere. In registering the oscillatory curve the steady deflection of the shadow magnified 1000 diameters was three cm. Adjusting the tension until a three cm deflection was produced by 1.59×10^{-8} ampere a photographic record was again made and found to be aperiodic. A slight increase in tension caused barely perceptible overshooting of the equilibrium position so that exact aperiodicity had been very approximately attained. It is thus evident that within the limits of error of the measurements the assumption that the ratio k/m is constant holds good over this range of tensions also. Determinations of τ_{02} at critical damping were made from curves registered with the string on film run at three meters/second, using the method described in Part 1, pp. 147–48. The value so obtained was 2.4×10^{-3} second, in good agreement with the calculated value.

APPENDIX D

COMPUTATION OF VALUE OF m

By rearrangement of Eq. (6), Part 1, we have $m = HI\tau_0^2/32U$. We have shown in Section 3, §3, that τ_0 is determinate and unique in spite of variation of k and of the effective value of m. Knowing the true value of τ_0, we can readily calculate the true value of m, the linear density of the string. In a particular experiment the following values were measured,

$H = 23{,}500$ gauss.
$I = 1.22 \times 10^{-5}$ ampere or 1.22×10^{4} cgs.
$\tau_0 = 9 \times 10^{-4}$ second.
$U = 3 \times 10^{-3}$ cm.

$$m = \frac{2.35 \times 10^{4} \times 1.22 \times 10^{-6} \times 8.1 \times 10^{-7}}{3.2 \times 3 \times 10^{-2}} = 2.42 \times 10^{-7}$$

$m = 2.42 \times 10^{-7}$ grams per centimeter length.

APPENDIX E

MAGNETIC FIELD DATA

To present any considerable part of the data which has been accumulated during the progress of experimental work connected with the development of the new designs which have been described in this paper would probably not be worth the space required. It seems worth while to present some comparative data regarding the energy expenditure required to produce a given field strength in several different types and some figures regarding leakage. The effect of shortening the airgap in Type B and C is well shown by the three magnetization curves in Fig. 9.

TABLE 2. *Watts consumed for production of field strengths stated.*

Field Strength in Gauss	Type A	Type B	Leyden Model Columbia copy	Cambridge Old Type	Edelmann Large Model
8,000	1.5	0.37	1.5	7.0	7.0
10,000	2.5	0.63	2.5	12.0	12.0
12,000	3.5	0.96	4.0	18.0	20.0
14,000	5.0	1.35	6.0	38.0	25.0
16,000	6.0	1.81	8.0	40.0	70.0
18,000	8.0	2.16	13.0	65.0	160.0
18,000*	9.0	2.35	15.0	78.0	240.0
20,000	11.0	2.94	22.0	125.0	.....
21,000	15.	3.37	33.0	226	.....
22,000	19.	3.84	55.0	422	.....
25,000	90.	11.75	125+	.....	.....
26,000	250.	24.0	.....	.....	.....
27,000	.....	58.0	.....	.....	.....
28,000	.....	143.8	.....	.....	.....

* The Edelmann instrument has windings concentrated over poles 8.5 cm in diameter. This makes the average length of a turn rather long and the resistance considerable. The experiment stopped at the last value given because the accumulation of heat was so rapid that the insulation was beginning to char.

The above table illustrates the great advantage of suitable design. The distributed windings of the Leyden Model, Type A and Type B produce the necessary flux quite as certainly as the concentrated winding of the Edelmann Model. In fact the design of this instrument is such that no advantage can be taken of the concentrated winding since the accumulated heat endangers the insulation long before saturation is reached. In the old type Cambridge instrument there was more room for windings and the outer half was wound of heavier wire. The heat seems to have been better dissipated also as it handled 422 watts

without charring the insulation. Without water cooling it would be impossible to work with any such expenditure of energy and that it is quite unnecessary is shown by the production of the same field strength in the Leyden type at 55 watts. The production of a field in type B with an expenditure of 3.84 watts equal to that which required 422 in the older Cambridge type is partly due to the distribution of the windings, but largely to the shortness of the airgap which lessens the reluctance and diminishes the leakage.

Einthoven calculated the leakage factor for the Leyden Model to be 10.9 at a field strength of 10,000 gauss. That is, the stray flux will be 10.9 times the useful flux at that field value. The accuracy of his calculation is attested by a measurement which the writer made on the

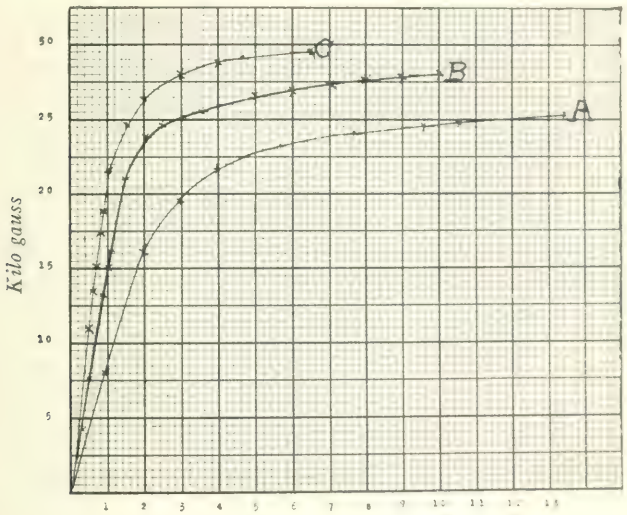

FIG. 9. *Magnetization curves for Types A, B, and C.*

Columbia copy at this field strength. The experimental value of the leakage factor was found to be 10.95. The leakage factor of Type B ranges from 5 at 10,000 gauss to about 8 at 27,000 gauss. The leakage factor in the old type Cambridge instrument must be very large, but I have never measured it.

Measurements of a Type B instrument show that the field strength in the region of the perforation averages about one third of the maximum value. In Type A and in the Leyden Model it is about one half the maximum value. The shorter airgap in Type B probably produces diminished leakage into the central region.

APPENDIX F

THE DAMPING COEFFICIENT

The damping coefficient, or coefficient of mechanical resistance, k, has been briefly considered in Section III, §4. In Section III, §2, the writer has referred to a theory developed by Stokes as probably explaining variations in the effective value of m which were observed by Einthoven. The theory of Stokes predicts that a cylinder of small diameter moving slowly in a viscous fluid will experience a large increase of mass and also of damping. Einthoven, after careful measurements concluded that k is constant over the entire working range of tensions. Regarding the precision of his measurements there is no question. Their interpretation rests, however, on the meaning of certain mathematical expressions derived from the equation

$$\ddot{u} + \frac{k}{m}\dot{u} + \frac{\mu}{m}u = 0 \tag{1}$$

Being an ordinary differential equation, this is strictly applicable only to a system with one degree of freedom, but for the purpose of the present discussion a more general treatment would merely complicate the expressions without adding anything of material interest. We shall not attempt to introduce the symbols employed by Einthoven (18) which are somewhat different from those used in these papers. The only new symbol in (1) is μ, which is here used to denote the coefficient of stiffness. When multiplied by the steady current deflection, U, the product, μU, is the restoring force which is in equilibrium with the impressed force during the continuance of steady deflection. The dimensions of μ are, therefore, $[MT^{-2}]$. In place of μ, Einthoven introduces $1/c$, where c is a coefficient of compliance or sensitivity.

Consider a photographic record of a steady current deflection, damping being either critical or more than critical. At the point of inflexion of the curve $\ddot{u}=0$. Introducing this value in (1), a solution for this point is at once obtained. This is essentially Einthoven's procedure. We have at the point of inflexion

$$u_i = -\frac{k}{\mu}\dot{u}_i \tag{2}$$

The negative sign may be disregarded as it refers only to direction of measurement. Of the quantities in (2), u_i is the ordinate of the curve at the point of inflexion and its value is read directly from the record.

The slope of the curve at the point of inflexion gives u, and $1/u$ is the sensitivity of the galvanometer which is readily determined, so that k appears to be known. Although rigorously u is zero only at a point, the velocity does not change rapidly in the immediate neighborhood of this point and it is conceivable that for a short stretch in this immediate neighborhood the motion might depend on an approximate equilibrium between the impressed force and the mechanical resistance. As stated, Einthoven finds k constant over the entire range of non-oscillatory motion by this method and, employing another method, he obtains almost the same value for oscillatory motion. He has reported one experiment, however, which can hardly be reconciled with this view of the matter. This experiment, already noticed in Section III, consisted in recording a deflection of the galvanometer at two sensitivities differing tenfold. The current was so adjusted as to give the same deflection in each case and for the lesser sensitivity the speed of the photographic plate was made ten times that at which the record of greater sensitivity was taken. The resulting curves are published in his 1903 paper (1), Figs. 1 and 3 of Plate III. They are of almost identical shape. The mathematical interpretation of this experiment is that the damping, $k/2m$, has remained constant. The range of sensitivities in these experiments covers the condition where Einthoven found large variations of m, so that k must have varied between these two conditions otherwise the ratio would not have been constant. The constancy of this ratio for small tensions of the string is decided by this experiment with a high order of precision and in Appendix C the writer has described an experiment which shows that k/m is still constant up to tensions which approach the limit of safe operation. While in general k and m for a cylinder moving in air would not vary in such a way as to preserve constancy of their ratio, nevertheless Stokes' theory indicates that for certain values of the argument this may occur.

As it appears impossible that k should be constant and equally impossible that Einthoven could have failed to detect the change which theory predicts will be large, the suspicion is aroused that perhaps Eq. (2), which appears to give the value of k, may for some reason not apparent on the surface, conceal the variations. Since the interpretation of the results of measurement depends entirely on Eq. (1), it is admissible to seek the answer to our question by analytical method. As the interest centers about the point of inflexion, we shall commence by obtaining mathematical expressions for the ordinate of the curve at

this point, the time at which it occurs and the velocity at the point. We shall obtain these data first for the case of critical damping, for which the solution is (Part 1, Appendix f)

$$u = Ue^{-\Delta t}(1+\Delta t) \tag{3}$$

in which for brevity $\Delta = k/2m$. Differentiating (3), we obtain for the velocity,

$$\dot{u} = -U\Delta^2 te^{-\Delta t} \tag{4}$$

The negative sign in (4) occurs because (3) is written in the form which defines a decaying deflection. Differentiating a second time, we have for the acceleration,

$$\ddot{u} = -\Delta^2 e^{-\Delta t}(1-\Delta t) \tag{5}$$

The remark regarding the sign of (4) applies also to (5). The condition (other than $t = \infty$), for (5) to vanish is obviously $t = 1/\Delta$, which gives the time at which the point of inflexion will occur.

We must now obtain corresponding values for the overdamped condition. The solution of (1) for damping more than critical is (Part 1, Appendix h),

$$u = U\left\{e^{\beta_1 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right) - e^{\beta_2 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right)\right\} \tag{6}$$

where $\beta_1 = -\Delta + \sqrt{\Delta^2 - \mu/m}$ and $\beta_2 = -\Delta - \sqrt{\Delta^2 - \mu/m}$. Differentiating (6), the velocity is,

$$\dot{u} = U\left\{\beta_1 e^{\beta_1 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right) - \beta_2 e^{\beta_2 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right)\right\} \tag{7}$$

Differentiating (7) the acceleration is,

$$\ddot{u} = U\left\{\beta_1^2 e^{\beta_1 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right) - \beta_2^2 e^{\beta_2 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right)\right\} \tag{8}$$

The criterion (other than $t = \infty$) for (8) to vanish is,

$$\beta_1^2 e^{\beta_1 t}\left(\frac{\beta_2}{\beta_2-\beta_1}\right) - \beta_2^2 e^{\beta_2 t}\left(\frac{\beta_1}{\beta_2-\beta_1}\right) = 0\,.$$

Solving this for t, we readily obtain,

$$t = \frac{1}{\beta_2-\beta_1}\log\frac{\beta_1}{\beta_2} \tag{9}$$

Noticing that

$$\frac{\beta_2}{\beta_2-\beta_1} = 1 + \frac{\beta_1}{\beta_2-\beta_1},$$

(6) may readily be put into the form,

$$u = U\left\{e^{\beta_1 t} + \left(\frac{\beta_1}{\beta_2-\beta_1}\right)\left(e^{\beta_1 t} - e^{\beta_2 t}\right)\right\} \tag{6a}$$

Substituting the value of t from (9) in (6a), we obtain

$$u_i = U\left\{e^{\log\frac{\beta_1}{\beta_2}\frac{\beta_1}{\beta_2-\beta_1}} + \left(\frac{\beta_1}{\beta_2-\beta_1}\right)\left[e^{\log\frac{\beta_1}{\beta_2}\frac{\beta_1}{\beta_2-\beta_1}} - e^{\log\frac{\beta_2}{\beta_1}\left(\frac{\beta_1}{\beta_2-\beta_1}+1\right)}\right]\right\} \quad \text{whence}$$

$$u_i = U\left\{\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}} + \left(\frac{\beta_1}{\beta_2-\beta_1}\right)\left[\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}} - \frac{\beta_2}{\beta_1}\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}}\right]\right\}, \tag{9}$$

$$u_i = U\left\{\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}} + \left(\frac{\beta_1}{\beta_2-\beta_1}\right)\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}}\left(1-\frac{\beta_1}{\beta_2}\right)\right\}, \text{ or,}$$

$$u_i = U\left\{\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}}\left(1+\frac{\beta_1}{\beta_2}\right)\right\} \tag{10}$$

From (7), giving t its value in (9),

$$\dot{u}_i = U\left\{\left(\frac{\beta_1\beta_2}{\beta_2-\beta_1}\right)\left[e^{\log\frac{\beta_1}{\beta_2}\frac{\beta_1}{\beta_2-\beta_1}} - e^{\log\frac{\beta_2}{\beta_1}\left(\frac{\beta_1}{\beta_2-\beta_1}+1\right)}\right]\right\}$$

whence,

$$\dot{u}_i = U\left\{\left(\frac{\beta_1\beta_2}{\beta_2-\beta_1}\right)\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}}\left(1-\frac{\beta_1}{\beta_2}\right)\right\}$$

or,

$$\dot{u}_i = U\beta_1\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_2}} \tag{11}$$

Eq. (2) may be written as an explicit statement of the value of k,

$$k = \mu u_i/\dot{u}_i \tag{12}$$

Critical damping is defined by the relation $\Delta^2 = \mu/m$, or $\mu = m\Delta^2$. Then from (3) and (4),

$$\mu u_i/\dot{u}_i = \frac{m\Delta^2 U e^{-\Delta t}(1+\Delta t)}{-U\Delta^2 t e^{-\Delta t}} = \frac{m(1+\Delta t)}{-t}$$

Whence, putting $t = 1/\Delta$ for the point of inflexion,

$$\mu u_i / \dot{u}_i = -2m\Delta = k$$

The significance of the negative sign as before is due to writing (3) in the form for a decaying deflection. We arrive at an equation which we might write, ignoring the sign, $k/2m = \Delta$, which at first sight suggests a mere identity. We shall show that Eq. (12) leads to the same result when applied to the overdamped condition. In this condition Δ still has the same constant value, but μ has diminished, say to μ_b.

From (10) and (11) we have,

$$\frac{\mu_b u_i}{\dot{u}_i} = \frac{\mu_b U\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_1}}\left(1+\frac{\beta_1}{\beta_2}\right)}{U\beta_1\left(\frac{\beta_1}{\beta_2}\right)^{\frac{\beta_1}{\beta_1-\beta_1}}}$$

which immediately reduces to,

$$\frac{\mu_b u_i}{\dot{u}_i} = \frac{\mu_b(\beta_1+\beta_2)}{\beta_1\beta_2} \tag{13}$$

From the values of the β's we at once find $\beta_1+\beta_2 = -2\Delta$ and $\beta_1\beta_2 = \mu_b/m$, and substituting these values (13) becomes

$$\frac{\mu_b u_i}{\dot{u}_i} = -2m\Delta = k,$$

as was shown for the just aperiodic condition.

For the oscillatory case Einthoven obtained a value of k by determination of the damped period and the logarithmic decrement. (See Eqs. (8) and (9), Appendix B.) If we take the decrement for a whole period,

$$k = 2m\lambda/\tau_k \tag{14}$$

From Eq. (1), Appendix B, $\lambda = \Delta\tau_k$. Substituting this value in (14), we have $k = 2m\Delta$.

Although $k/2m = \Delta$ suggests an identity, it is not difficult to show that these equal ratios are by no means identical. Let Δ_a be the ratio for a string carrying a load of moving air and consider the case of critical damping. $\Delta_a^2 = \mu/m$. Should the m in this equation be also regarded as loaded with moving air or is it the true mass of a unit length of the string? The physical meaning of μ/m is perhaps best apprehended from the equation,

$$\tau_0 = \frac{2\pi}{\sqrt{\frac{\mu}{m}}} \qquad (15)$$

In (15) τ_0 is the free period *without damping* and the mass which corresponds to the period without damping is obviously the true mass. So we might write the equation $k = 2m\Delta$,

$$\Delta = \Delta_a$$

where the first Δ corresponds to true mass and minimum value for the resistance coefficient. Similar considerations apply to the overdamped condition of which critical damping is merely a special case. Regarding the m in Eq. (14), it is to be noticed that Einthoven derived this from the free period without damping and the sensitivity data of steady current deflection with the string in equilibrium. This is also a value of true m. So Einthoven should have obtained exactly the result he did, constancy of the value of k for all tensions, since in all these equations the loaded values of k and m are never separated. The Eq. (1) was based on the assumption of constant values for these quantities and would not describe the motion of the string galvanometer even approximately were it not for the fact that Δ rather than its components is of importance. In investigating points on the curve of motion away from the point of inflexion, Einthoven puts the equation into a form such that k and m are separated. Here he at once experiences difficulty. Believing as a result of the measurements we have just discussed that k is constant he is forced to ascribe the entire discrepancy to variation of m.

We have shown that the finding of a constant value for k at all velocities of the string flows from the form of the equations by means of which the experimental results were treated. Properly interpreted these results do not appear inconsistent with the Stokes theory, on the contrary they support it. We may also remark that the result of the present investigation evidences the precision of Einthoven's experimental work.

It is perhaps to be regarded as fortunate that the ratio Δ, rather than the coefficient, k, should have turned out to be constant since this renders it possible to use Δ in calculation and obtain correct results even though the components of Δ may vary in a manner not easily determinable.

APPENDIX G

ON THE PREPARATION OF FINE QUARTZ FIBERS

Aside from their use as the supporting core of galvanometer strings, fine quartz fibers are an important laboratory commodity. As many laboratory workers seem to be unfamiliar with a simple and convenient method by which they have been made for many years at the Columbia laboratory, it seems advisable to add a note on that subject. The bow and arrow method of making them described by Boys (33) is excellent for producing very long fibers, but requires much experience in preparation and handling of the equipment if fibers smaller than .003 mm are wanted. Nichols (34) described a method of blowing short fibers and Wertheim-Salomonson (8) described an elaboration of this method which permits of securing fibers long enough to serve as galvanometer strings. All of these methods have required a room flooded with sunshine to enable the operator to see and catch the fiber. Some ten years ago the construction of a new building adjacent to the writer's laboratory made sunshine unavailable except for about one hour daily and this led to the development of a method of working by artificial light which has proved more satisfactory than the use of natural sunlight. This method is a blowing process, but fibers more than a meter long can be produced and their fineness seems limited only by the patience and skill of the operator. Fibers .0015 mm in diameter are readily obtained by a reasonably good laboratory technician with a little practice. It is also possible to make much larger fibers. I have not tried to find out how large, but .01 mm presents no difficulty.

As raw material one may use the fused silica of commerce. Either the "silky" or transparent rods will produce good fibers. The type of oxyhydrogen lamp is important. Most of these will be found to be of the blow-through type with an inner tube for oxygen running out to the tip of the burner. While these can be used it is extremely difficult to adjust them and keep them in such adjustment that they will blow fibers with certainty. On the other hand a good mixing type of burner requires no adjustment and with very little practice gives uniform success. An excellent mixing burner was formerly supplied by Newton of London as part of a calcium light equipment and this I have found to serve admirably. Many of the older laboratories probably have these burners. Fig. 10 is a sketch of the illuminating apparatus. It is a wooden box one meter high, open at the top in the middle third. The middle part of the back and the bottom are covered with dead black fabric such as is used for light-proof window shades. The inside surfaces

of the front, sides, roof and lateral parts of the back are painted with white enamel. Six 75 watt nitrogen filled lamps arranged three on a side, furnish the light and give an intense, but diffuse illumination in

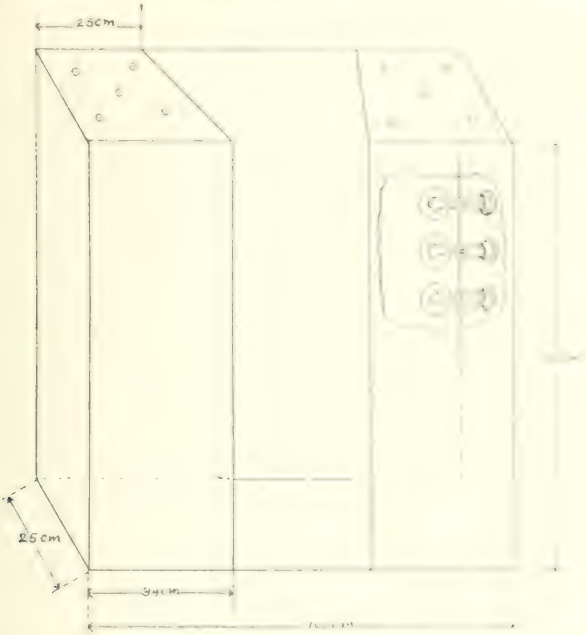

FIG. 10. *Sketch of illuminating box used in making quartz fibers.*

the middle third. At the back of the middle third is hung a piece of black velvet reaching the full height. There is a rather strong upward current of air in the middle of this box and in front of it due to heat

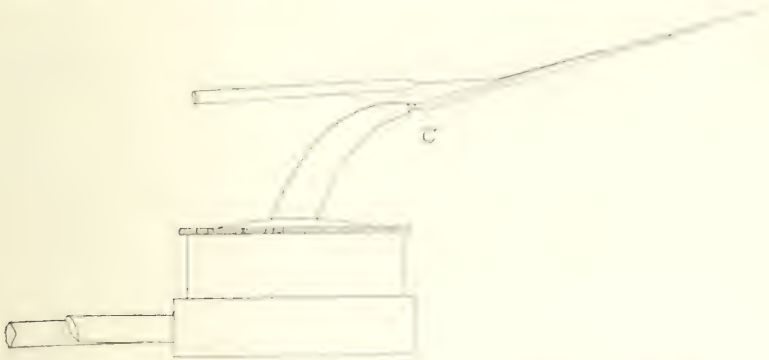

FIG. 11. *Method of blowing quartz fibers. "C" is the position of the "hot spot". The quartz softens, but does not fuse in the tip of the flame and is pulled out as the operator lowers his hand the rodlet melts when it reaches the hot spot.*

from the burner and the lamps and this air current serves a useful purpose as will presently appear.

The hot spot of the burner is at *C*, Fig. 11. A piece of rod is heated in the hot spot and drawn out to a thin, needle-like rodlet which is pulled apart at the middle. Each piece can be used to make a fiber. The size of the rodlet can be varied and to some extent determines the size of the fiber produced. The small end is introduced into the point of the flame as shown in Fig. 11 and the end toward the hand lowered until a considerable length of the rodlet is in the flame. Here again the length of rodlet in front of the hot spot determines to some extent the size of the fiber. The rodlet does not melt in the front part of the flame, though it softens and is drawn out straight by the moving hot gas. As soon as a part of the rodlet enters the hot spot it melts and the anterior part is shot like an arrow by the stream of gas across to the velvet where it will probably stick. If the hand is held steady, the fiber does not immediately break and the ascending current of hot air wafts it upward in a loop so that in this way fibers as long as two meters are sometimes drawn, though one meter is more usual. When it finally breaks the air current straightens out the loop and the operator may reach forward and grasp the rodlet and so secure the fiber. Sometimes the rodlet fails to stick. If the fiber is long it may be suspended in the air or blown up very slowly so that it can be caught. If short it will probably be blown upward so rapidly that it will escape. These events happen with a rapidity which is hardly conveyed by the description.

The flame should be smooth and almost noiseless and may be as long as ten cm. It is best to get oxygen in a cylinder at high pressure and use a reducing valve of the best quality. Illuminating gas is usually used with the oxygen. Heating quartz repeatedly in this flame causes a change which may be recognized by its losing its smooth ductility. As soon as this tendency is noticed, break off and discard the ends of the rods which have been heated. Do not allow the rods to lie where they will be contaminated while hot and do not handle the parts which are to be heated. The fibers will not come in contact with the velvet ordinarily and they should not touch any part of the apparatus as otherwise they may become contaminated which is important if they are to be coated. There are numerous other small details, but anyone working a few hours will learn them for himself.

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